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IDENTIFICATION OF CRITICAL BIOLOGICAL PARAMETERS AFFECTING GASTROINTESTINAL ABSORPTION

John M. DeSesso
Richard D. Mavis

The Mitre Corporation
Civil Systems Division
7525 Colshire Drive
McLean, Virginia 22102-3481

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HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY
HUMAN SYSTEMS DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

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FOR THE COMMANDER

MICHAEL B. BALLINGER, Lt Col, USAF, BSC
Chief, Toxic Hazards Division
Harry G. Armstrong Aerospace Medical Research Laboratory

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An analysis of absorption-related anatomical and physiological features of the gastrointestinal tract and the mechanisms of absorption of both nutrients and nonnutrients has been performed in order to identify critical parameters affecting gastrointestinal absorption. This analysis identified the following parameters as likely to have a major effect on the kinetics of gastrointestinal absorption: vascularity, transit time, surface area, contents, enterohepatic cycling, and the chemical properties of the compound studied. A search of the scientific literature for published pharmacokinetic data on 15 selected low molecular weight, volatile halogenated hydrocarbons was conducted in order to obtain information on the importance of the critical parameters in the pharmacokinetic description of the gastrointestinal absorption of these chemicals. The available data showed effects of gastrointestinal contents, transit times, and chemical properties on the gastrointestinal absorption of these chemicals. Experimental approaches to more quantitatively describing these effects and determining the impact of the other critical parameters on the gastrointestinal absorption of

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22b. TELEPHONE (Include Area Code)
(513) 255-2704

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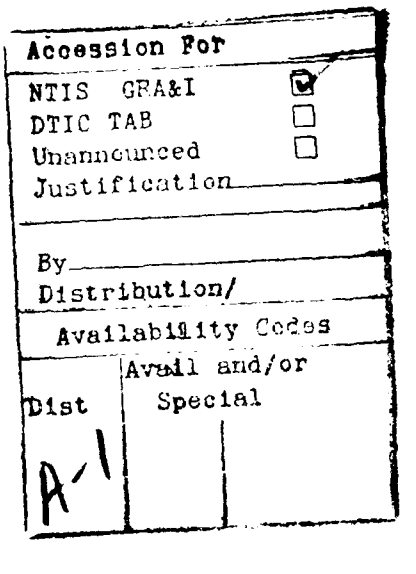

EXECUTIVE SUMMARY

INTRODUCTION

The Air Force is involved in a very large task of hazard management through its Installation Restoration Program. The pioneering efforts of the Armstrong Aerospace Medical Research Laboratory (AAMRL) in the development of extrapolation-based pharmacokinetics have the potential to provide critical technical support for enhancing the accuracy of risk assessments at hazardous waste sites, thereby enhancing the effective use of resources in the restoration of the sites.

The AAMRL has successfully modeled the pharmacokinetics of inhaled volatile organic compounds, using equations and constants based on separately characterized physiological events. Current efforts are extending this approach to the modeling of compounds ingested orally and absorbed in the gastrointestinal (GI) tract. The complexity of the GI tract makes a physiological description of GI absorption a more complicated task than the description of the absorption of inhaled compounds. The AAMRL has obtained the assistance of The MITRE Corporation in identifying critical biological parameters to be considered and compared between species in a physiological description of GI absorption of nonnutrients. This task was accomplished by a comprehensive survey of published literature concerning the anatomy and physiology of the human and rat GI tract as well as mechanisms of absorption of nutrients and nonnutrients. From this survey, a list of critical parameters affecting absorption has been derived from absorption data as well as from first principles of biology and chemistry. The impact of these parameters on the gastrointestinal absorption of 15 volatile halogenated hydrocarbons was evaluated on the basis of analysis of available published pharmacokinetic data. Experimental approaches to a more quantitative description of the role of the identified parameters in gastrointestinal absorption were suggested.

ABSORPTION-RELATED FEATURES OF THE GASTROINTESTINAL TRACT

The gastrointestinal tract is an epithelium-lined tunnel of the environment through the body. In terms of the processing of ingested substances in the alimentary canal, the gastrointestinal tract can be divided into those regions that serve as conduits through which material is propelled (mouth, pharynx, esophagus, and rectum) and those regions that digest and absorb substances (stomach, small and large intestines). The epithelium and underlying connective tissue (mucosa) of the absorbing regions of the alimentary canal exhibit several specializations that enhance the transfer of substances from the lumen of the alimentary canal to the vascular system. These include both modifications of the epithelial surface to increase its surface area (folds or plicae; depressions or crypts; villi; and microvilli) and a rich supply of blood and lymphatic capillaries.

The major site of absorption of orally ingested substances is the proximal small intestine (duodenum and jejunum). Absorption also occurs in the remaining small intestine, the colon, and the

stomach. Under certain conditions, the epithelium under the tongue can absorb materials, but the quantity of materials absorbed at this site is negligible.

When the dimensions and surface areas of the absorbing regions of the gastrointestinal tracts from humans and rats are compared, several interesting findings emerge. While the small intestine (particularly the jejunum) makes up a larger proportion of the intestinal tract in rats than in humans, the relative surface area of the human small intestine is five times greater than that of the rat.

In the case of poorly absorbed substances, the amount of time that the substance is in contact with the absorptive epithelium will affect the extent of its absorption. The period of time that elapses as chyme traverses a particular region of the alimentary canal is the transit time. In humans, transit times for various regions of the alimentary canal range from seconds (in the mouth, pharynx and esophagus) to days (in the large intestine). Transit time in the human stomach ranges from 6 minutes (for water on an empty stomach) to 5 or more hours (after a fatty meal). In the small intestine, transit time for both humans and rats is approximately 3 to 4 hours.

The nature of the material in the lumen changes as the material traverses the alimentary canal. This is due to the dispersion of the ingested material in secreted digestive juices, action of enzymes, presence of bacterial flora, and the absorption of fluid and nutrients from the lumen in various regions. In addition, the nature of the contents itself can affect both transit time and absorption. High fiber content tends to decrease the transit time and to sequester some lipophilic substances leading to decreased absorption of some substances.

The small intestines of humans and rats are vascularized by both blood and lymphatic capillaries. Although the blood perfusion rate is about 1,000 times that of the lymphatics, about 20 percent of absorbed material is transported by the lymphatics during maximal absorption. This is important because the blood vascular route delivers absorbed materials to the liver for metabolism whereas the lymphatic route bypasses the liver and enters the venous system near the heart. In addition to the differences in sites of connection to the systemic circulation, there are differences in the substances that are transported by the two routes. Most of the chylomicrons (formed from digested lipids) are too large to traverse the fenestrations of the blood capillaries and are transported by way of the lymphatics. The differential routes of absorption for chylomicrons and water soluble materials may lead to partitioning of absorbed substances between those that are metabolized by the liver immediately and those that bypass the liver.

THE MECHANISMS OF GASTROINTESTINAL ABSORPTION OF NONNUTRIENT CHEMICALS

The gastrointestinal epithelium presents a water-insoluble lipophilic barrier to the uptake of materials from the intestinal lumen. Passive diffusion is the most likely mode of transport of nonnutrient chemicals across the GI epithelium. The rate of absorption by passive diffusion will be influenced by the solubility, size, and ionization state of the chemicals. It is unlikely that nonnutrient

chemicals will be transported by carrier proteins designed to carry nutrients. These proteins are very specific and generally do not bind chemicals other than the ones they normally transport. Some exceptions to this have been observed, including the transport of nonnutrient metals by calcium and iron carriers. In addition, the absorption of certain amphoteric β-lactam antibiotics, which have structural similarities with amino acids, has been shown to follow saturable kinetics, consistent with their transport by amino acid carrier proteins.

VEHICLE EFFECTS ON GASTROINTESTINAL ABSORPTION

The rate of absorption of a chemical from the GI tract can be greatly influenced by the other materials present in the GI lumen. In GI absorption experiments with laboratory animals, a relatively small amount of the chemical of interest is often administered as a dilute solution in a solvent such as water or oil. The nature of the solvent used in such an experiment can affect the rate or extent of uptake of the chemical and can result in uptake that is very different from that which occurs when the normal ingestion of food accompanies the ingestion of nonnutrients. The vehicle in which a nonnutrient compound is dissolved or suspended can have a marked effect on the rate of absorption. Oil persists for hours in the GI tract and, when used as the vehicle, produces relatively slow and irregular absorption of lipid-soluble compounds. Water is rapidly absorbed from the GI tract and essentially deposits dissolved compounds in a concentrated form on the GI epithelium, resulting in more rapid absorption. In contrast to the effect of oil on lipid-soluble compounds, the absorption of poorly-soluble griseofulvin and water-soluble heparin were enhanced by an oil in water emulsion compared to administration in water alone.

When associated with a large portion of food, a lipid-soluble compound is absorbed over the course of hours, as compared to instantaneous absorption when dosed in a few µl of alcohol. This probably reflects the partitioning of the compounds between the luminal contents and the GI epithelium. Food has less of an impact on rate of absorption of relatively insoluble compounds such as antibiotics. Osmotic gradients have been shown to have effects on absorption consistent with the osmotically-induced flow of water in the intestine, but not in the stomach.

The effect of vehicle on the uptake of trace nonnutrients from the GI tract can have a major impact on the pharmacokinetic disposition of the nonnutrients. Slower uptake from the GI tract in response to vehicle differences has been demonstrated to result in maximum blood concentrations that were an order of magnitude lower than those resulting from more rapid absorption. Also, the site of absorption can depend on whether the compound is deposited in an empty stomach, in which case a lipid-soluble compound will be quickly absorbed in the stomach, or is associated with food, which limits its contact with the GI epithelium and allows considerable passage along the GI tract before complete absorption occurs. In addition, the opportunity for metabolism in the GI tract is greatly enhanced by a vehicle that slows the absorption of the chemical.

CRITICAL PARAMETERS IN THE MODELING OF ABSORPTION

The following six parameters were identified as likely to be critical in the modeling of GI absorption: transit time and motility, GI contents, surface area, vascularity, enterohepatic cycling, and the chemical and physical properties of the chemical being absorbed. Not all of these parameters will be important for all chemicals, but a definitive knowledge of the role that each of these plays in absorption of a chemical of interest should allow a quantitative description of the absorption process.

IMPACT OF THE CRITICAL PARAMETERS ON THE ABSORPTION OF VOLATILE HALOGENATED HYDROCARBONS

Published pharmacokinetic data available in the published literature for oral administration of volatile halogenated hydrocarbons containing one or two carbon atoms were evaluated for evidence regarding the impact of the identified six critical parameters on GI absorption of these compounds. The kind of data needed for this type of evaluation was found to be scarce. From the data available, a significant impact of three of the six parameters was evident. The effects of GI contents were most evident. Transit times, as they are affected by GI contents, also play an evident role in GI absorption of these chemicals. Similarities in the GI absorption of the different chemicals for which data were available and physical properties that they have in common also show that the physical properties of chemicals being absorbed are important determinants of the rate and extent of GI absorption.

SUGGESTED EXPERIMENTAL APPROACHES TO EVALUATING THE IMPACT OF PARAMETERS ON GI ABSORPTION

Early measurements of the concentrations of the chemical of interest in blood, different sections of the GI tract, lymph, bile, exhaled and esophageal air after oral administration would allow a more complete definition of the role of the critical parameters in the GI absorption of these compounds.

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SECTION 1

INTRODUCTION

The Toxic Hazards Division of the Armstrong Aerospace Medical Research Laboratory (AAMRL) at Wright-Patterson Air Force Base is a leading laboratory in the development of quantitative descriptions of the physiological and biochemical events involved in the uptake, distribution, metabolism, and elimination of toxic agents by living organisms. Originally known as physiologically-based pharmacokinetics, this approach represents an advance from conventional pharmacokinetics. With the conventional pharmacokinetic approach, experimental data are fitted to a mathematical equation containing the minimum number of terms and constants needed to produce a fit. Conventional pharmacokinetic constants are derived by fitting an equation to pharmacokinetic data. The resulting equation and the constants derived from the fitting process do not contain specific information about the individual biological events involving the compound studied. The equation is, rather, a composite abstract mathematical description of several different occurrences.

Using a physiologically-based pharmacokinetic approach, in contrast, a series of equations is developed that reflects actual events such as absorption, blood flow, metabolism, and equilibration of the chemical within various tissues. The constants in these equations are independently derived from physiological and biochemical measurements. The goodness of fit between the physiological equations and the pharmacokinetic data reflects the accuracy with which the events are chosen and described, and better fits are obtained by adding previously undescribed events or obtaining more accurate parameters to describe the known events. This approach tests the extent of knowledge of the events that are involved in the uptake, distribution, metabolism, and elimination of toxic chemicals. Since the tissue concentrations of toxic agents are among the values described by this approach, the extrapolation of tissue-specific doses between different routes of exposure and between species can be made on the basis of independently measurable physiological parameters. The enhanced accuracy of extrapolation afforded by this approach has resulted in its designation as extrapolation-based pharmacokinetics.

The estimation of dose from information provided in an exposure scenario for comparison with a reference dose is one of the most critical elements of risk assessment. Because extrapolation-based pharmacokinetics promises a new level of confidence for the extrapolation of a tissue-specific dose between species and between routes of exposure, it will bring a new dimension of accuracy to this difficult but essential task in the management of toxic hazards. The Air Force is involved in a very large task of hazard management through its Installation Restoration Program. The pioneering efforts of the AAMRL in the development of extrapolation-based pharmacokinetics have the potential to provide critical technical support for enhancing the accuracy of risk assessments at hazardous waste sites, thereby enhancing the effective use of resources in the restoration of the sites.

The AAMRL has successfully modeled the pharmacokinetics of inhaled volatile organic compounds, using equations and constants based on separately characterized physiological events. Current efforts are extending this approach to the modeling of compounds ingested orally and absorbed in the gastrointestinal (GI) tract. The complexity of the GI tract makes a physiological description of GI absorption a more complicated task than the description of the absorption of inhaled compounds. The AAMRL has obtained the assistance of The MITRE Corporation to identify critical biological parameters to be considered and compared between species in a physiological description of GI absorption of nonnutrients.

This task consisted of two phases. In phase I, relevant parameters were identified. In phase II, the influence of the identified parameters on the GI absorption of halogenated hydrocarbons containing one or two carbon atoms was evaluated from available published data.

The approach to phase I of this project was to survey, in a comprehensive manner, published literature concerning the anatomy and physiology of the human and rat GI tract as well as mechanisms of absorption of nutrients and nonnutrients. From this survey, a list of critical parameters affecting absorption has been derived from absorption data as well as from first principles of biology and chemistry.

Phase II was accomplished by a survey of the literature for articles that might contain pharmacokinetic data on GI absorption of 15 halogenated hydrocarbons. The compounds used in this

search are listed in appendix C. Relevant articles were analyzed for information on the importance of the critical parameters on the GI absorption of this class of compounds.

Section 2 of this report is a summary of critical anatomical and physiological features related to absorption. Section 3 defines the potential mechanisms for the absorption of nonnutrients from the GI tract. Section 4 discusses the effects of vehicle on GI absorption. Section 5 lists the critical parameters identified in phase I. Section 6 presents the results of phase II, a survey and analysis of the published literature for data which provide information on the importance of the critical parameters in GI absorption of volatile halogenated hydrocarbons containing one or two carbon atoms. Section 7 contains suggested experimental approaches to further defining the role of critical parameters in GI absorption of volatile halogenated hydrocarbons. In section 8, references cited as sources of specific information or studies are listed separately from monographs and advanced texts from which more general information was derived. Appendix A contains detailed information on the anatomy and physiology of the organ systems of the GI tract, and appendix B describes the mechanisms of absorption of nutrients. Appendix C is a list of the halogenated hydrocarbons surveyed for data in phase II.

SECTION 2

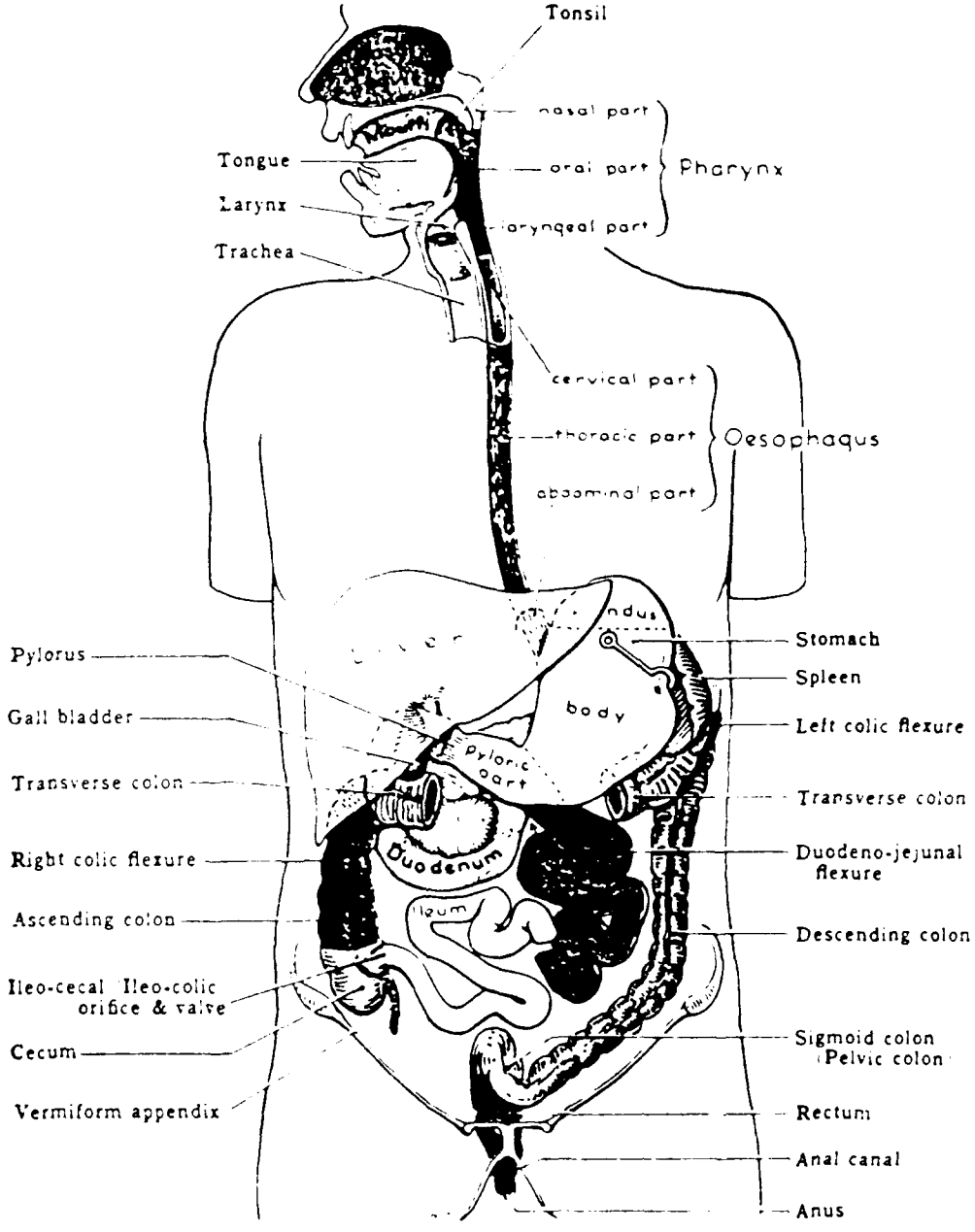
ABSORPTION-RELATED FEATURES OF THE GASTROINTESTINAL TRACT

The gastrointestinal tract is essentially an open ended, epithelium-lined tube that extends from the mouth to the anus. If it is viewed as such, it becomes clear that the lumen and its contents are outside the body; the lumen of the gastrointestinal tract is a tunnel of the environment through the body. The gastrointestinal tract itself serves as an interface between the environment and the circulatory system. The main functions of the gastrointestinal tract are the digestion of food and the absorption of nutrients. The structure of the absorbing regions of the gastrointestinal tract is well-suited for this task. The purpose of this section is to discuss the anatomical and physiological features of the gastrointestinal tract that are related to absorption of nutrients and nonnutrients in both humans and rats.

2.1 THE NATURE OF THE BARRIER TO ABSORPTION

From the mouth to the anus, the alimentary canal (depicted in figure 2-1) is lined with a mucous membrane, or mucosa, that serves as the first barrier to entry of materials into the body. The mucosa is comprised of an epithelial sheet underlain with a thin layer of loose connective tissue (lamina propria) that contains both blood and lymphatic capillaries. Absorption of a substance from the lumen of the alimentary canal requires that the substance pass through the epithelium, a portion of the lamina propria, and the walls of the blood or lymph capillaries.

The alimentary canal can be subdivided by function into those regions that serve primarily as conduits to propel ingested substances through the canal (mouth, pharynx, esophagus, lower rectum, and anal canal) and those that function primarily to digest and absorb the substances (stomach, small and large intestines). The mucosa of the conduit portions of the alimentary canal generally exhibits a moist stratified squamous epithelium and a lamina propria that is not richly vascularized. The topography of these regions is generally flat with few of the surface irregularities that increase the mucosal surface area. Due to the multiple layers of epithelial cells and the relatively sparse vascularization of the lamina propria, this type of mucosa is not well adapted to the ready transfer of



Source: Anderson, 1978.

Figure 2-1
Diagram of the Digestive System

substances from the lumen to the vascular system. In contrast, the mucosa of the absorbing portions of the alimentary canal consists of a simple columnar epithelium with a prominent and richly vascularized lamina propria. The mucosa in the absorbing regions of the alimentary canal exhibits a variety of modifications that increase the surface area including folds, depressions (crypts), and finger-like projections (villi). This increased surface area of the mucosa is conducive to the transfer of substances from the lumen to the vascular system.

Although the epithelia of the absorbing regions of the alimentary canal are not composed of a uniform population of cells, there is one cell type that is important in the absorption of materials from the lumen and is the predominant cell in all absorbing regions. This cell type is referred to as an enterocyte. Enterocytes are columnar epithelial cells that are bound to their neighboring cells at the luminal surface by terminal bars (tight junctions). Their apical cell membranes possess numerous microvilli (estimated at 3,000 to 7,000 per cell) which serve to increase the surface area available for absorption by an estimated factor of 20. The presence of these microvilli on the enterocytes creates the appearance of the brush border that is seen with the light microscope. The absorptive epithelia possess a carbohydrate-rich glycocalyx coat on the surface of the microvilli, and they rest on a basement membrane.

The lamina propria in the absorptive regions of the alimentary canal possesses a rich supply of blood and lymphatic capillaries. When villi are present, the lymphatic capillaries are slightly dilated, blind-ending tubes that occupy the center of the villus. The blood capillaries form a network of vessels beneath the basement membrane.

At various portions of the absorbing regions of the alimentary tract, absorption is favored by mucosal structures such as fenestrations in the endothelial cells of the capillaries or pores of different sizes in the membranes of the enterocytes.

The third structure that comprises the mucosa is a thin layer of smooth muscle that underlies the lamina propria. This is termed the muscularis mucosae. The muscularis mucosae is not present in all regions of the alimentary canal. In regions of active absorption such as the small intestine, it is well developed and even extends up into the central cores of the villi. The function of this muscular

layer appears to be related to the rhythmic movements of the villi that agitate the layer of intestinal secretions and chyme that are in contact with the epithelium. It is thought that this helps to promote the absorption of fatty acid micelles and some water soluble nutrients. It is noteworthy that some *in vitro* methods have been used to test the ability of substances to be absorbed through the intestinal mucosa by determining the amount of material that traverses an excised preparation of small intestinal wall. Such preparations are denervated and the muscularis mucosae is not active. Consequently, the absorption of substances may be hindered by the unstirred layer next to the epithelium and their *in vivo* absorption may be underestimated. The mechanisms by which substances traverse the mucosa will be discussed in detail later in this document.

2.2 SITES OF ABSORPTION

While the systemic absorption of orally ingested materials takes place mainly in the small intestine and colon (particularly in the duodenum and proximal jejunum), absorption can and does take place at other sites along the alimentary canal. The most important nonintestinal site of absorption is the stomach, which is capable of absorbing nonionized, lipophilic molecules of moderate molecular size. A second, but quantitatively less important, site of potential nonintestinal absorption is in the mouth, in the region under the tongue. Even for substances that possess optimal physical and chemical properties for absorption, the extent of absorption in the mouth is limited by the length of time that the substance is in contact with the mucosa in that region.

2.3 DIMENSIONS AND SURFACE AREAS

The primary site of absorption of substances from the lumen of the alimentary canal is the intestinal tract. Table 2-1 presents a comparison of the absolute and relative lengths of the intestinal tract and its major subdivisions in humans and rats. The length of the human intestinal tract is only about five times the length of the rat intestinal tract, despite the much larger body size of the human (70 kg) compared to the rat (0.25 kg). The relative sizes of the subdivisions of the intestinal tracts also differ. In rats, the small intestine comprises 85 percent of the total length of the intestinal tract and about 90 percent of the small intestine is jejunum. In contrast, the small intestine of humans is only 76 percent of the total intestinal tract length and only 38 percent of the small intestine is

Table 2-1
Comparison of the Length of the Intestinal Tract and
Its Major Subdivisions in Humans and Rats

Region of Intestinal Tract	Human			Rat		
	Length (cm)	% Total	% Subdivision	Length (cm)	% Total	% Subdivision
Total Intestinal Tract	660[a]	100	--	120-170[b]	100	--
Small Intestine	500	76	100	102-145	85	100
Duodenum	25		5	9.5-10		8
Jejunum	190		38	90-135		90
Ileum	285		57	2.5-3.5		2
Large Intestine	160	24	100	22-26	15	100
Cecum	7		4.5	5-7		25
Colon	98		61	9-11		42
Rectum	55[c]		34.5	8		33

[a]Assembled from data in Snyder et al., 1975.
[b]Assembled from data in Hebel and Stromberg, 1986.
[c]Includes both sigmoid colon and rectum for comparison to analogous region in rat.

jejunum. Another outstanding difference between the intestinal tracts of the two species is the relative sizes of the ceca. In rats, the cecum accounts for approximately 25 percent of the length of the large intestine, whereas the cecum accounts for only about 4.5 percent of the length in humans.

While the comparison of the absolute and relative lengths of the subdivisions of the rat and human intestinal tracts reveals some interesting differences between the two species, it does not provide a complete picture of the surface areas available for absorption. The luminal surface of the human intestine exhibits an elaborate complement of structural features that serve to increase its absorptive surface area per unit length of intestine compared to the rat. Table 2-2 presents a comparison of the absolute and relative surface areas for various regions in the gastrointestinal tracts of rats and humans.

Despite the fact that the human small intestine is only 5 times the length of the rat small intestine, the absolute absorptive surface of the human small intestine is 200 times that of the rat small intestine. In both species, the majority of the surface area is found in the jejunum. In the case of rats, this is due to the relative length (90 percent of the small intestine) of the jejunum, whereas in humans the large surface area is due to the presence of numerous folds of mucosa (plicae circulares) that populate the jejunum.

Due to the great difference in size between rats and humans, these data are not directly comparable; a way must be found to normalize physiological data in order to account for the anatomical and physiological differences between the human and the rat. In order to do this, it is necessary to consider the differences in metabolic rates of the two species. One method of accounting for metabolic differences among humans (Pinkel, 1958) and between human and animal species has been to normalize on the basis of the surface area of the body (cf. Calabrese, 1983, for discussion). The ratio of the surface area of a region of the gastrointestinal tract to the surface area of the body is termed the relative surface area. Comparison of the relative surface areas of the human and rat small intestines reveals that humans have a 5 times greater relative surface area for the small intestine than rats. In the case of the jejunum, which is the site of greatest absorption in both species, the difference is not quite so large, but jejunal relative surface area in humans is still 3.8 times that of rats. Since the amount of a substance that crosses the enteric mucosa is determined by its flux (amount of mass per

Table 2-2
Comparison of the Absolute and Relative Surface Areas of the Absorptive Regions of the Gastrointestinal Tracts of Humans and Rats

Region	Human		Rat	
	Absolute Surface Area (m2)	Relative Surface Area (Region/Body)	Absolute Surface Area (m2)	Relative Surface Area (Region/Body)
Body	1.85	--	0.045	
Stomach	0.0525	0.03	TBF[a]	TBF
Small Intestine	200	108	1	22
Duodenum	19[b]	10.3	0.08	1.8
Jejunum	138.6[b]	74.9	0.90	19.8
Ileum	42.4[c]	22.9	0.02	0.4

[a]TBF = To be found.
[b]Calculated using the data in Snyder et al., 1975, and proportion of mucosal surface area to length of intestine as 98:1.
[c]Calculated using the data in Snyder et al., 1975, and proportion of mucosal surface area to length of intestine as 20:1.

unit surface area per unit time), the impact of this increased relative enteric surface area in humans on the comparative absorption of substances is twofold. First, substances that are equally well absorbed in both rats and humans are likely to be absorbed more quickly in humans (i.e., exhibit a higher rate of absorption). Second, substances that are poorly or incompletely absorbed by both species are likely to be absorbed to a greater extent by humans.

Table 2-2 also indicates the small absolute surface area of the human stomach (0.0525 m^2) compared to the small intestine (200 m^2). This 3,800-fold difference in surface areas helps to explain the reason that gastric absorption of substances is generally small when compared to enteric absorption.

2.4 MOTILITY AND TRANSIT TIME

Most of the alimentary canal of both humans and rats is surrounded by at least two layers of smooth muscle. The muscle fibers of the inner layer are arranged circumferentially relative to the lumen; those of the outer layer are arranged parallel to the long axis of the canal. The coordinated, rhythmic contractions of these layers of smooth muscle cause the intestinal motility which is responsible for the thorough mixing of chyme, the continual rejuxtaposition of chyme with the brush border of the enterocytes, and the propulsion of food through the GI tract in a net aboral direction (peristalsis). In addition to the external layers of smooth muscle, there is a thin layer of smooth muscle in the lamina propria (muscularis mucosae) that causes the intestinal villi to undulate, thereby agitating the layer of fluid (the unstirred layer) that is associated with the brush border of the enterocytes.

Transit time is the amount of time taken for a bolus of food or chyme to traverse a region of the gastrointestinal tract. In the mouth, transit time is determined by voluntary control over the length of time spent in chewing. Once a bolus of food is passed to the pharynx and esophagus, control of transit time is governed by both gravity and primary peristalsis. The total transit time through the human pharynx and esophagus is about 6 seconds. Upon reaching the stomach, ingested materials experience different transit times that depend upon the nature of their contents. Transit time in the human stomach can be as brief as 6 minutes for water on an empty stomach or as long as 5 or more

hours for a fatty meal. Generally, meals comprised of the various dietary constituents empty from the stomach at varying times with carbohydrate meals emptying first, protein meals at an intermediate time, and fatty meals last. Liquids drunk during a meal frequently bypass the solid portions of a meal and enter the duodenum quickly. Early digestion of dietary fats in the stomach results in the presence of long chain fatty acids (optimal carbon chain length of 14) in the stomach and duodenum. These long chain fatty acids inhibit the release of the enteric hormone gastrin and this effect is the apparent mechanism for the delay in gastric emptying of a fatty meal.

Chyme traverses the human small intestine at a rate of 1 to 4 cm per minute. The velocity of transport is faster in the proximal portions of the small intestine (duodenum and proximal jejunum) and decreases as chyme approaches the ileum. In the usual case, chyme traverses the entire small intestine in 3 to 4 hours. Transit time for chyme in the human large intestine is considerably slower. Depending upon the amount of fiber or other insoluble materials in the diet, transit time through the large intestine in healthy humans is 2 to 4 days.

The time for chyme to traverse the small intestine of rats is also approximately 3 to 4 hours. As in the case of humans, the velocity of transport is faster in the proximal segments of the small intestine than in the distal segments. It should be noted that the time for chyme to traverse the rat jejunum is longer than in humans because the jejunum accounts for 90 percent of the rat small intestine. It should be noted, however, that the values reported for transit times in both humans and rats are subject to great variations depending upon many factors including health status, age, and fasting state. For this reason these values should be determined experimentally for modeling purposes rather than accepting published values.

Most gastrointestinal absorption of substances takes place during the 4 hours that chyme is in the small intestine. Absorption of fluid and electrolytes, as well as products of bacterial digestion of otherwise indigestible materials, takes place during the 4 days that chyme remains in the large intestine. Some absorption of readily absorbed substances does take place in the stomach, but on a quantitative basis the amount of nutrients that undergo gastric absorption is generally low compared to the amount absorbed enterically.

Generally, an increase in intestinal transit time will increase the absorption of poorly absorbed or incompletely absorbed substances. However, this is not always true. For instance, some substances (e.g., anticholinergics) increase transit time by inhibiting intestinal smooth muscle motility. While inhibition of peristalsis does increase intestinal transit time, it also inhibits the movements of the intestine that mix chyme and agitate the unstirred layer of fluid. These movements are essential for the absorption of lipophilic substances because, without them, the unstirred layer forms a barrier between the brush border membrane and the chylomicrons that contain the lipophilic substances.

2.5 NATURE OF LUMINAL CONTENTS

The nature of the luminal contents in both humans and rats is altered as the contents traverse the alimentary canal. The alteration is due to (1) the physical dispersion of ingested solid matter by chewing and the muscular activity of the stomach coupled with the mixing of the ingested matter with imbibed fluid and digestive juices, (2) the action of enzymes that cleave nutrients into readily assimilated molecules, (3) the absorption of nutrients and fluid from the lumen, and (4) the addition of bacterial flora to the contents.

Table 2-3 presents a summary of the daily volumes and pHs of digestive fluids secreted by the various portions of the human digestive tract and adnexal organs. It should be noted that of the approximately 10 liters of fluid that enter the upper digestive tract each day (1.5 to 2.0 liters of ingested fluid plus 8.0 to 8.5 liters of secreted digestive juice), only 1.5 liters enters the large intestine and only about 100 to 150 ml of water is found in the daily output of feces (Granger et al., 1985). While the preceding numbers are striking, it must be recalled that they are for the net flow of fluid. Depending upon the osmolarity of the luminal contents, there may be considerably greater flow of fluid into and out of the lumen due to convection (see section B.2.1). Since most of the secreted fluids are isotonic with plasma, the convection is caused by the osmotic activity of the ingested contents and not by the digestive juices themselves.

The pH of the luminal contents is modified by the pH of the various secretions. In most regions of the digestive tract, the secretions are slightly alkaline and the luminal contents exhibit a

Table 2-3
Daily Volume and pH of Human Digestive Juices[a]

Source	Secretion	Daily Volume (ml)	pH
Salivary Glands	Saliva	1,200	6.0-7.0
Stomach	Gastric Juice	2,000	0.8-3.5
Exocrine Pancreas	--	1,200	8.0-8.3
Liver/Gall Bladder	Bile	700	7.8
Small Intestine	Succus Entericus	3,000	7.8-8.0
Brunner's Glands	--	50 (?)	8.0-8.9
Large Intestine	--	60	7.5-8.0
Total		8,210	

[a]Modified from Guyton, 1971.

pH of 7 to 8. The stomach is the lone exception to this general statement. The secretion of acid by the gastric mucosa results in acidification of chyme. In humans, the pH of the chyme in the stomach may be as low as 1 to 2; in rats, the pH is less acidic (pH 4.5 to 5.0). When chyme enters the duodenum, it is quickly changed by the alkaline secretion of the Brunner's glands. The pH of the chyme affects the ionization state of certain molecules and, therefore, can affect absorption. The role of ionization on absorption of substances is discussed in section 3.1.2.

Bacterial flora populate much of the gastrointestinal tract of both humans and rats and become an ingredient of the luminal contents. In rats, large numbers of microorganisms are found in the stomach, small intestine, and colon. In humans, however, microorganisms are virtually absent in the stomach and proximal small intestine; large numbers of bacteria are not encountered until the chyme reaches the colon. Since the bacteria are living organisms that are capable of metabolizing substances in the chyme, they can alter the contents and affect absorption. In addition, the difference in geographical location of bacteria within the digestive tracts of rats and humans can affect the site and extent of absorption of some substances. Once the chyme has reached the colon, bacteria become a major component of the luminal contents. With the dehydration of the chyme that occurs as it traverses the colon bacteria eventually make up over 33 percent of the dry weight of the luminal contents in the distal human colon (Granger et al., 1985).

Other characteristics of the luminal contents that impact absorption are due to the nature of the ingested material itself. For instance, diets that are high in fiber tend to (1) sequester some of the poorly soluble and lipophilic substances making them unavailable for contact with the brush border (Gregus and Klaassen, 1986) and (2) decrease transit time through the intestinal tract (Granger et al., 1985). Both of these tendencies favor decreased absorption from the lumen.

2.6 BLOOD AND LYMPH FLOW

The tissues of the human and rat alimentary canals, like other tissues of the body, contain vessels of both the blood and lymphatic vascular systems. Both vascular systems return their contents to the systemic circulation, but they differ with regard to their routes of return. The blood vasculature from the stomach, small intestine and colon coalesce into veins that are tributaries of the hepatic

portal vein. All blood from the hepatic portal vein flows into the sinusoids of the liver where it is in intimate contact with the liver tissue before re-coalescing into one of the hepatic veins that drain into the inferior vena cava en route to the heart. In contrast, the lymphatic vessels from these organs drain into the sac-like cisterna chyli (the origin of the thoracic duct). Lymphatic fluid flows via the thoracic duct to join the venous blood returning to the heart at the juncture of the left subclavian and left internal jugular veins in the root of the neck. This route of return completely bypasses the liver. The first capillary bed that will be traversed by the material distributed via the lymphatic route is in the lungs.

While the rate of perfusion for blood vasculature is about 1,000 times that of the lymphatic vasculature for most tissues (Granger et al., 1985), the flow of lymph from the small intestine is measurable. The resting rate of lymphatic flow in humans is 0.095 ml/min/100 g of intestinal tissue (Jacobson, 1985); this flow rate increases during the physiological hyperemia that occurs after meals. When the intestine is absorbing at near maximum rates, about 20 percent of the absorbed fluid is transported by the lymphatic vasculature (Jacobson, 1985).

The absorbed material that is transported by both the blood and lymphatic vessels passes through the enterocytes and is found in the interstitial fluid of the lamina propria. The interstitial fluid has a dual origin. It is derived both from fluid that passes out of the lumen of the alimentary canal and also from an exudate of plasma originating from the proximal portions of the capillaries in the lamina propria. This exudate is increased by the physiological hyperemia occurring in the presence of chyme. The movement of absorbed material from the interstitial fluid to the vessels of the two vascular systems depends on the relative fluid uptake by the two systems as just described and also on the anatomical arrangement and structure of the vascular beds described (See figure 2-2).

The blood capillaries in the lamina propria of the alimentary canal form a network (plexus) that is located immediately subjacent to the basement membrane of the epithelial layer of enterocytes. The walls of the blood capillaries are formed by a single, thin endothelial cell layer and the basal lamina that surrounds it. The distance between the basement membrane of the enterocytes and the blood capillaries is no more than a few microns. Not only are these vessels located close to the epithelia, but also there are openings in the endothelial cells (fenestrations) that are overlain only by

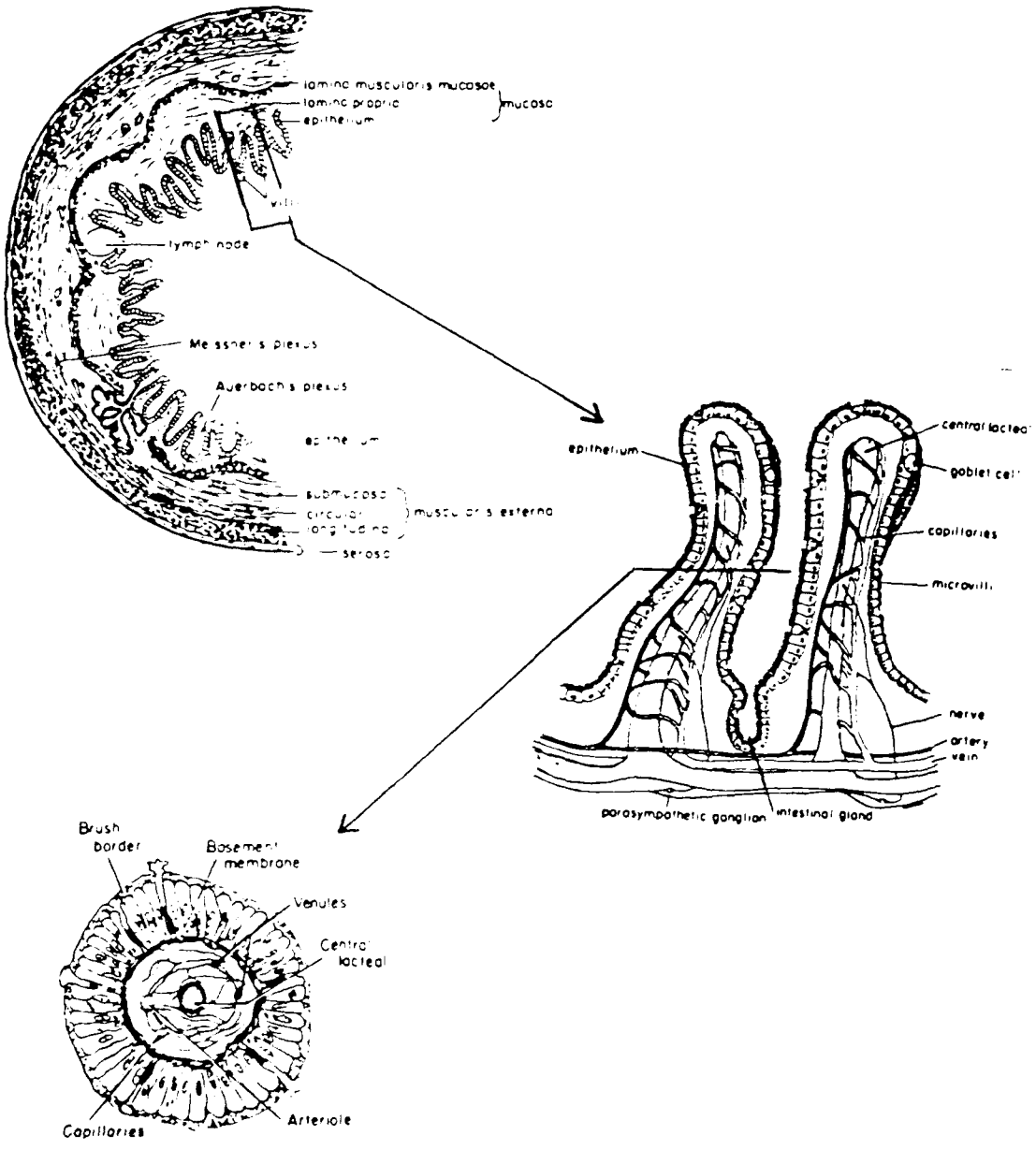


Figure 2-2
Illustration of the Arrangement of Blood and Lymphatic Vessels in the Small Intestine

the basal lamina. These fenestrations appear similar to portholes through the cell and serve to expedite the absorption of water and various water-soluble materials. The size of the fenestrations is estimated to be 500 Å in diameter (Bloch and McCuskey, 1973), although proteins as small as albumin (~75 Å diameter) are excluded from absorption, presumably due to the sieving action of the basal lamina.

In contrast, the lymphatic channels of the alimentary canal (as exemplified by the small intestine) consist of single sac-like vessels (the lacteals) which are located deeper in the lamina propria than the blood capillaries. In the small intestine, the lacteals occupy the center of the villi; the distance between the basement membrane and the lacteal is estimated to be 50 microns (Jacobson, 1985). The walls of the lymphatic vessels are also comprised of a single endothelial cell layer. However, the lymphatic endothelial cells are thinner than the corresponding blood capillary endothelial cells, there is no overlaying basal lamina, and there are no fenestrations. Despite the lack of fenestrations, the lymphatic vessels are permeable to even the largest lipid particles derived from the diet (~6,000 Å diameter chylomicrons as described in appendix B.2.4.; Granger et al., 1985) due to the presence of permeable spaces (apertures) between adjacent endothelial cells (Weiss, 1973).

These differential routes of absorption for chylomicrons and water soluble materials may lead to the partitioning of absorbed substances between the lymphatic route (which bypasses the liver) and the blood vascular route (which delivers all of its absorbed substances to the liver). Substances that are soluble in water will follow the blood vascular route; substances with high lipophilicity and low water solubility may associate with the chylomicrons and therefore follow the lymphatic route; and amphipathic substances with moderate lipophilicity may partition between the chylomicrons and the aqueous route.

Many lipophilic nutrients (e.g., long chain fatty acids, cholesterol, vitamins) are absorbed via the lymphatics of the intestines. The total amount of most xenobiotic compounds absorbed via the lymphatics under normal physiological conditions is small. This is related to the great disparity between lymphatic and blood perfusion rates in intestinal tissues (DeMarco and Levine, 1969). However, the intestinal lymphatics are a major route of absorption for some high molecular weight, lipophilic xenobiotic substances. For substances that possess these physical and chemical

characteristics, it is important to determine the extent to which they partition between the blood and lymphatic systems. Among the xenobiotic substances that exhibit major partitioning into intestinal lymphatics are several confirmed and suspected carcinogens including benzo(a)pyrene (Rees et al., 1971), aminostilbene derivatives and 3-methylcholanthrene (Kamp and Neumann, 1975), and DDT and its lipophilic derivatives (Sieber et al., 1974; Sieber, 1976). Following oral administration, benzo(a)pyrene appeared in thoracic duct lymph within 20 minutes (Rees et al., 1971) and aminostilbene derivatives appeared within 5 minutes. In most cases, concomitant ingestion of lipid increased the rate of uptake of these substances (Sieber et al., 1974). Fractionation of recovered lymph revealed that most of the substances travelled within the core of the chylomicrons (Sieber et al., 1974).

Although the early appearance of xenobiotic substances in intestinal lymph demonstrates that lymphatic absorption should be considered when modeling gastrointestinal absorption, several caveats should be noted. The lymphatics are a portion of the body water compartment. Consequently, substances that are absorbed via the portal (blood) vascular system will equilibrate into the water portion of the lymph and will find their way into the thoracic duct lymph. It is estimated that 10 percent of the lymph in the suprahepatic thoracic duct in the rat is contributed by the liver (Mann and Higgins, 1950, via Kamp and Neumann, 1975). This means that lymph should be collected from infrahepatic portions of the thoracic duct, cisterna chyli, or mesenteric ducts at early times after administration. Further, if there is doubt as to the source of the xenobiotic materials, the chylomicrons should be separated from the infranatant (water) fraction of the lymph, and the location of the substances within the lymph should be determined. Most lipophilic substances absorbed via the intestinal lymphatics are expected to be located within the chylomicrons.

2.7 ENTEROHEPATIC CYCLING

The hepatic portal system of both humans and rats is comprised of blood flow from the capillaries of the small intestine, through the tributaries of the hepatic portal vein, to the sinusoids of the liver without traversing the heart. Venous blood from the small intestine, containing absorbed substances, is said to have undergone enterohepatic circulation when it has traversed this route. Once in the liver, many absorbed substances are removed from the blood by the liver parenchymal cells.

Xenobiotic substances are frequently biotransformed in the liver cells. The products of biotransformation are generally larger and more polar than the parent compounds. These modifications favor excretion into bile ducts and decrease reabsorption in the intestine after the metabolites are secreted with bile into the GI tract. The net result is that for such compounds, the bile serves as a route of fecal excretion. This mode of excretion limits the entry of the excreted compounds into the general circulation and the distribution to other tissues. Certain compounds, however, may be reabsorbed from the GI lumen subsequent to enterohepatic absorption and biliary excretion, either in the absence of biotransformation or as a result of degradation of metabolites in the intestinal lumen. Re-entry of these compounds into the enterohepatic circulation and re-excretion into bile may then occur, possibly over a number of cycles of these two events. This process is known as enterohepatic cycling.

SECTION 3

THE MECHANISMS OF GASTROINTESTINAL ABSORPTION OF NONNUTRIENT CHEMICALS

The GI epithelium presents a water-insoluble lipophilic barrier to the uptake of materials from the intestinal lumen. This section describes the potential mechanisms for absorption of nonnutrients across the GI epithelium. The mechanisms by which nutrients cross the epithelium are described in appendix B.

3.1 PASSIVE DIFFUSION

Passive diffusion is the most likely mode of transport for nonnutrient chemicals. The rate of absorption by passive diffusion will be influenced by the solubility, size, and ionization state of the chemicals.

3.1.1 Solubility

The solubility of a chemical will have a major influence on the rate of passive diffusion across the GI epithelium. Since the barrier is composed of lipid-soluble components, the diffusion of water-soluble components across the barrier is slower than the diffusion of lipid-soluble components. Water itself permeates the epithelium several-fold more rapidly than water-soluble components, apparently through aqueous pores. These pores are impermeable to all but the smallest of water-soluble compounds, however. Relatively small water-soluble components, such as sodium or potassium ions, appear to have access to aqueous pores and can traverse the GI epithelium, although at rates that are several orders of magnitude less than water. Water-soluble compounds with molecular diameters greater than about 5Å would be expected to be absorbed very slowly through the lipid membranes and may not be completely absorbed before passing through the entire GI tract. Lipid-soluble compounds, in contrast, are freely soluble in the lipid membrane barrier and will be readily absorbed if they can attain contact with the cell surface. The most rapidly absorbed compounds will be those with significant solubilities in both lipid and water. Such compounds, for example, ethanol, can diffuse up to the membrane surface through the aqueous contents of the intestinal lumen, dissolve in the cell surface membrane, and pass through it. Compounds that are only soluble in lipid and not in

water will be dependent upon the presence in the GI tract of mixed micelles, the products of the digestion of dietary lipid, for presentation to the lipid membrane after penetration through the unstirred layer and mucous coating. Compounds that are sparingly soluble in either water or lipid will be most poorly absorbed and are likely to pass through the GI tract with little, if any, absorption.

3.1.2 Ionization State

Ionization state can affect the solubility of a compound and therefore its diffusion through a lipid membrane. A weak organic acid may be lipid soluble in the nonionized acid form and yet not permeate lipid membranes in the ionized, anionic form. For such a compound, absorption would be more favored in the stomach, pH 2, than in the intestine, where the pH is 7.5 to 8.0. The impact of pH on absorption, however, is minimized by the fact that, as the fraction of a compound that is in the nonionized form is absorbed, the remaining compound will rapidly equilibrate, such that molecules in the nonionized form are continuously available for absorption. The net movement of a weak acid from lumen to submucosa will be enhanced by the higher pH of the submucosa which will serve to trap the compound in the ionized form. The absorption of a weak organic base, in contrast to an organic acid, would be favored by a higher pH, where the unionized form would predominate. As with an organic acid, rapid equilibrium between the ionized and nonionized forms would occur and allow continuous absorption even at a pH where the ionized form predominates.

3.2 ROLE OF SPECIFIC NUTRIENT CARRIERS

It is unlikely that nonnutrient chemicals will be transported by carrier proteins designed to carry nutrients. These proteins are very specific and generally do not bind chemicals other than the ones they normally transport. Some exceptions to this have been observed. Thallium is transported by iron-binding proteins, for example, and the calcium carriers bind and transport lead. The relatively simple structure of metal ions makes these substitutions possible.

Organic nutrients, whose structures are more complex than metal ions, will be transported by carrier proteins that are likely to be more specific, and recognition of nonnutrient chemicals by these proteins is less likely than by metal ion carriers. It is, therefore, very unlikely that carriers that normally transport organic nutrients will transport nonnutrient chemicals. An example of a carrier protein that exhibits a high degree of specificity is the carrier that mediates the sodium-coupled active

transport of glucose. This protein also transports galactose, whose structure is virtually identical to glucose. Other sugars, however, with less similar structures, are not transported by this carrier, and it seems highly unlikely that a nonnutrient chemical would be transported by the glucose carrier protein. Carriers of various amino acids appear to be less specific, since four different carriers transport a total of 23 different amino acids. It is conceivable that some nonnutrient compounds might have structures similar enough to amino acids that they could be recognized by the binding sites of these carriers and be transported by them. The concentration dependence of the absorption by the rat small intestine of certain amphoteric β-lactam antibiotics, which have structural similarities with amino acids, has been shown to follow saturable kinetics consistent with their transport by amino acid carrier proteins (Tsuji et al., 1981a, 1981b).

3.3 PINOCYTOSIS

Pinocytosis is a possibility for the entry of particles into the enterocytes. Particles of azo dye and of latex are believed to be taken up by this mechanism, followed by exocytosis into the interstitium and entry into the lymphatics.

3.4 ENTEROHEPATIC CYCLING

While the process of enterohepatic cycling is not a mechanism of absorption, the extent to which a compound is cycled by the enterhepatic system can greatly influence uptake and excretion. The extent of cycling of a compound depends upon the relative efficiency of absorption from the intestine and reexcretion into the bile. Total absorption from the intestine and only partial reexcretion will result in the uptake of the compound into the organism over the course of cycling. Incomplete absorption coupled with complete reexcretion in bile would result in total excretion of the compound without distribution to the rest of the organism. The enterohepatic cycle may also serve as a mechanism of excretion for a body burden of a compound that is secreted with bile and not completely reabsorbed by the intestine. Generally, polar compounds of molecular weight greater than 350 tend to be excreted in the bile. Large species differences in enterohepatic absorption have been reported (Gregus and Klaassen, 1986).

SECTION 4

VEHICLE EFFECTS ON GASTROINTESTINAL ABSORPTION

The rate of absorption of a chemical from the GI tract can be greatly influenced by the other materials present in the GI lumen. In GI absorption experiments with laboratory animals, a relatively small amount of the chemical of interest is often administered as a dilute solution in a solvent such as water or oil. The nature of the solvent used in such an experiment can affect the rate or extent of uptake of the chemical and can result in uptake that is very different from that which occurs when the normal ingestion of food accompanies the ingestion of nonnutrients. This section discusses selected experimental evidence that illustrates the potential effects of the solvent vehicle on GI absorption, the possible mechanisms for such effects, and the implications for extrapolation modeling of GI uptake.

4.1 OIL VERSUS WATER

One of the most extensive studies comparing absorption of chemicals after gavage in water or oil was conducted by Withey et al. (1983). These authors administered the four similar chlorinated hydrocarbons, dichloromethane, chloroform, dichloroethane, and trichloroethylene, to fasted rats by intragastric gavage of solutions of these compounds at equal concentrations in 3 to 5 ml of water or corn oil. They compared the total area under the curve of blood concentration versus time, the elimination rate constants, the peak blood concentrations, and the time to peak blood concentrations. The generally consistent finding was that the chemicals were absorbed more slowly and to a lesser extent from oil solutions than from water solutions.

Withey et al. (1983) calculated the ratios between the total area under the curve of blood concentration over time for water versus oil as the gavage vehicle. The respective ratios were 1.25, 8.71, 3.75, and 166 for dichloromethane, chloroform, dichloroethane, and trichloroethylene. The relatively large difference in the effect of vehicle on the absorption of trichloroethylene corresponded to a water solubility of this chemical that was about an order of magnitude less than the other chemicals. The peak blood concentrations were 2.8-, 6.5-, 5.3-, and 14.7-fold higher for water solutions of dichloromethane, chloroform, dichloroethane, and trichloroethylene compared to oil solutions. The time required to reach peak blood concentrations was in the range of 3 to 16 minutes

and was 3.1-, 1.1-, and 3.3-fold longer when methylene chloride, chloroform, and dichloroethane were dissolved in oil compared to water solutions. In addition, the blood concentration curves after gavage with oil solutions were very irregular in shape. One or more upward pulses followed the initial peak concentration over the 300 minute collection of data. Generally, blood content decreased more slowly than in the curves following absorption from water. The time required to reach peak blood concentrations of trichloroethylene administered in oil was not calculated because of the highly irregular shape of the time curve for this exposure. The data collected in this study did not allow a direct calculation of the overall extent of absorption, and the authors did not attempt such a calculation. Other studies of these chemicals, however, resulted in data that were consistent with complete absorption of these chemicals even from oil (Dekant et al., 1986; Brown et al., 1974).

The consistently slower absorption of these lipid-soluble low molecular weight chemicals from oil solution compared to water suggests that their greater solubility in oil decreases the rate of the movement of these compounds into the brush border membrane of the gastrointestinal epithelium. The relatively low solubility of these compounds in water would result in a partitioning between luminal water and epithelial membrane that would greatly favor the lipid membrane. With oil as the luminal vehicle, however, a partition coefficient of about one would be expected. In addition, contact between the oil phase and the lipid phase of the brush border membrane would be hindered by the aqueous glycocalyx coating of the microvilli, the overlaying layer of mucus, and possibly by the unstirred layer of water, all of which would be less penetrable by an oil phase than by a water phase. Another difference would be that the several ml of gavaged water would be absorbed relatively quickly (in a few minutes) (see appendix B), increasing the luminal concentration of the organic chemicals and increasing their rates of absorption. Absorption of oil, in contrast, requires a relatively long process (an hour or two) of emulsification, hydrolysis, and micelle formation, before absorption can occur. Thus, oil remains in the lumen for hours, and, in fact, the authors noted a considerable amount of oil in the feces, indicating incomplete absorption of the oil vehicle. The persistence of oil in the gastrointestinal tract throughout its transit would clearly favor longer retention of the hydrocarbons in the lumen compared to the situation where the chemicals were administered in a water vehicle.

The appearance of the peak blood concentration within 16 minutes or less with either water or oil as the vehicle suggests that the major initial absorption of the chemicals from oil did not involve digestion of the oil vehicle, which would have taken considerably longer. The occurrence of sporadic later peaks in the blood concentration curves after gavage of the oil solutions could reflect enhancement of absorption by the slower and relatively sporadic events involved in the digestion and absorption of oil. The authors suggested that the irregularities in the uptake from the oil vehicle could be explained by fractionation of the gavaged dose either by the formation of immiscible oil globules that experienced differential contact with the gastric mucosa or fractional emptying of the oil from the stomach.

A large difference in GI absorption of dichloromethane administered to mice in oil or water was reported by Angelo et al. (1986a). These authors measured the dichloromethane content in blood and other tissues, including the GI tract, and also the rate of expiration of dichloromethane and its metabolic products, after intravenous or oral administration. The content of dichloromethane in the GI tract decreased rapidly to less than 3 percent of the administered dose 20 minutes after the compound was administered orally in water. Absorption was much slower following oral administration in corn oil. From 30 to 50 percent of the dose remained in the GI tract 20 minutes after dosing in corn oil, and these values decreased by 10 to 20 percent after two hours. Although these data cannot be compared directly with the data of Withey et al. because different measurements were made in the two studies (i.e., blood concentration versus amount remaining in the GI tract), the overall pattern of slower GI absorption of dichloromethane from corn oil than from water is consistent. Thus, an effect of vehicle on the GI absorption of dichloromethane has been demonstrated in both rats and mice.

In a similar pharmacokinetic study of dichloromethane in rats by Angelo et al. (1986b), 70 to 80 percent of the compound was absorbed from the GI tract 40 minutes after administration in water. This rate of absorption is intermediate between the rates of absorption of the compound from oil and water observed in mice (Angelo et al., 1986a). Unfortunately, the rate of absorption from oil was not determined in the rat study, so that the vehicle effect in rats cannot be confirmed by this article.

An effect of vehicle on the carcinogenicity (Jorgenson et al., 1985) and hepatotoxicity (Bull et al., 1986) of chloroform has been shown. Chloroform induced liver tumors in B6C3F1 mice when chronically administered by gavage in corn oil but not when chronically administered in drinking water at a similar dose level (Jorgenson et al., 1985). In a follow-up study, the hepatotoxicity of chloroform in the same strain of mice was markedly greater when it was administered by gavage in corn oil as compared to gavage in an aqueous solution (Bull et al., 1986). Increased toxicity of chloroform administered in corn oil is in contrast to the decreased rate of absorption from corn oil and demonstrates that rate of absorption does not necessarily correlate with toxicity. The authors concluded that the enhanced toxicity of chloroform in corn oil compared to water was not due to the vehicle-induced differences in pharmacokinetics of chloroform reported by Withey et al. (1983), but rather a result of combined interactive effects of chloroform and corn oil on the liver.

In an earlier study, Withey (1976) reported a markedly different time course of blood levels of styrene after oral administration to fasted rats in water and vegetable oil. Aqueous dosing yielded data that were virtually identical to those obtained after intravenous injection. The blood levels decreased biexponentially starting at 5 minutes after gavage of a dose of 3.2 mg in 10 ml of water. By 30 minutes, the level was approximately 10 percent of the 5 minute value and the three time points at 5, 10, and 20 minutes defined an exponential loss with a half-time of approximately 17 minutes. Later time points, collected up to 150 minutes, defined a second exponential term with a half-time of approximately 47 minutes.

Gavage of an approximately 10-fold larger dose of 33 mg of styrene in 2 ml of corn oil, in contrast, yielded a very different type of curve. Blood levels increased approximately 6-fold over the course of 100 minutes after dosing to a peak value that was only twice that of the 5 minute value in the aqueous dosing experiment, despite the 10-fold higher dose administered with corn oil. From 100 to 300 minutes after the corn oil gavage, blood levels decreased to a value of approximately 10 percent of the peak value. The areas under the blood concentration curves estimated by MITRE, after intravenous and oral dosing with both water and oil solutions were in approximately the same proportion to dose in all three experiments. This suggests that the absorption was complete in both oral experiments. Thus, it appears that oil as the dosing vehicle compared to water, has the same effect on styrene absorption as it does on the absorption of chlorinated methane and ethanes, namely,

a decreased rate of absorption and a longer period of time over which absorption occurs. A 10-fold larger dose given in oil, and a 5-fold difference in gavage volumes are confounding factors which could conceivably influence the uptake of styrene and present possible alternative explanations for the differences in absorption observed with the different vehicles. The vehicle effect remains the most likely interpretation of the data, however.

The data of Withey (1976) were analyzed in a more recent study of the pharmacokinetics of styrene (Ramsey and Andersen, 1984). These authors found that the data after administration in oil were complex and could not be described by equations that assumed either first order or zero order absorption from the GI tract into portal circulation. The data from administration in water, in contrast, were successfully simulated by assuming first order absorption from the GI tract to the liver.

The effect of vehicle on the absorption of urushiol, the allergenic component of poison ivy, was studied by Skierkowski et al. (1981). Urushiol is a dihydric phenol with a side chain of 15 or 17 carbons in length. It is insoluble in water, yet is absorbed so slowly that its absorption is incomplete after an oral dose. The authors studied its absorption from corn oil or ethanol by measuring the amount of radioactivity in urine and feces over time after oral gavage to fasted rats of a 1 mg dose of radiolabeled compound in 0.5 ml of vehicle. The compound was incompletely absorbed, judging from the recovery of a major portion of the dose in the feces. A cumulative total of 53 percent of the administered dose was excreted in feces after administration in ethanol; 35 and 50 percent, respectively, were recovered in feces by 24 and 48 hours after dosing. Administration in corn oil resulted in somewhat less apparent absorption. A cumulative total of 71 percent of the total dose was excreted in feces with 61 and 69 percent appearing by 24 and 48 hours. The larger percentage of the total cumulative dose which appeared in feces at 24 hours after a dose in corn oil suggests that intestinal motility may have been increased as a result of the corn oil ingestion and that the increased motility resulted in decreased absorption of the compound.

The contribution of enterohepatic cycling to the fecal content of urushiol was evaluated in a separate experiment in which the total biliary excretion of the compound was measured after an oral dose in ethanol. A total of approximately 9 percent of the dose of urushiol was recovered in bile. This is only a small fraction of the total amount that was recovered in feces, and this result confirms

that approximately 85 percent of the compound excreted after dosing in ethanol was never absorbed. The authors used this result to support a conclusion that the presence of compound in the feces was not, to a major extent, the result of biliary excretion, and that the larger amount of urushiol in feces after corn oil dosing was evidence for less absorption. This conclusion implies that biliary excretion after a corn oil dose would be similar to that measured after ethanol dosing, even though this parameter was not measured after dosing in corn oil. The authors did not consider the possibility that ingested corn oil could have an effect on biliary excretion. It is possible that the higher fecal content of urushiol in corn oil-dosed animals was in part a result of stimulation of biliary excretion by corn oil, which would return a greater portion of the absorbed compound to the fecal compartment. The extent of the contribution of increased enterohepatic cycling to the larger amount of urushiol in feces after corn oil dosing cannot be assessed without data on the effect of the corn oil vehicle on biliary excretion. Thus while a direct vehicle effect of corn oil directly on absorption is consistent with the data presented, an alternative effect on biliary excretion may also partly explain the difference in fecal excretion of this compound.

Corn oil as an emulsion in water was found to enhance the GI absorption in rats of the water-insoluble antibiotic, griseofulvin (Bates and Carrigan, 1975). The authors interpreted the zero order GI absorption of this compound to indicate that dissolution of the suspended particles of the drug was rate-limiting in absorption. The corn oil emulsion resulted in delayed onset of absorption, two-fold greater duration of absorption, two-fold greater maximum concentration, and approximately three-fold greater amount absorbed, as measured by the area under the curve of a plot of plasma concentration versus time, when compared with absorption of a suspension in water alone. These parameters of absorption were not significantly different in fasted and nonfasted animals. The authors did not speculate on the mechanism of the effects of corn oil on absorption of this compound. The results are consistent with an effect of the corn oil emulsion on maintaining the suspended antibiotic in a more dispersed form that presented more surface area for dissolution. Stimulation of the flow of bile into the duodenum by corn oil could also contribute to this effect, since bile could enhance the solubilization of the compound.

Intestinal absorption of the water-soluble sulfated polysaccharide heparin by rats and gerbils was also shown to be enhanced by intraduodenal administration in an emulsion of corn oil or

trioctanoin, but not mineral oil, compared to administration in water alone (Engel and Fahrenbach, 1968). The absorption of heparin was measured indirectly as the amount of heparin-releasable lipase activity in the blood and by clotting time, which is increased by heparin. No increase in clotting time or in lipase activity was observed when heparin was administered in water or in a mineral oil emulsion. This is consistent with the impermeability of the GI tract to this this relatively large (MW 14,000) ionized compound. Administration with an emulsion of a digestible oil, however, produced a significant increase in lipase activity and an approximately 10-fold increase in clotting time over control. The involvement of pinocytosis of the oil particles and the absorption of digested lipid were suggested by the authors as possible mechanisms of the enhancement. The lack of effect of mineral oil indicates that pinocytosis is not the sole explanation for the enhancement and that hydrolysis of the lipid is required.

4.2 GAVAGE VERSUS DIET

The normal vehicle present in the GI tract is ingested food. In many of the above studies, the animals were fasted before the administration of the test dose. This creates a situation suitable for comparing the effects of vehicle alone, but the data cannot necessarily be considered representative of absorption of a nonnutrient ingested along with a normal daily ingestion of food. There are a number of mechanisms whereby food can affect the absorption of co-ingested nonnutrients (Welling, 1977). The presence of food in the GI tract increases the flow of blood and lymph in the intestinal region, increases intestinal motility, stimulates the circulation of bile, and decreases the rate of gastric emptying. In addition, food can interact directly with nonnutrients, binding them or otherwise affecting the diffusion of compounds to the epithelial surface. All of these factors can have a marked effect on intestinal absorption. Welling (1977) has reviewed the effects of food on the GI absorption, by human subjects, of 55 drug products and preparations. The absorption of all but four of the drugs was affected by the presence of food. The predominant effect of food on drug absorption is inhibition or delay. An experimental distinction between a delay in absorption and a reduction in the total amount absorbed is often difficult. Generally the serum concentration of the drug reaches a maximum at a later time and at a lower concentration when the dose is accompanied by food. The absorption of a few drugs is actually enhanced by food.

A most marked effect of food on the GI absorption of a nonnutrient by experimental animals was reported by Pekas (1974). The absorption in rats of radiolabeled naphthyl N-methylcarbamate (carbaryl), a lipid-soluble insecticide, was studied when a dose of 7.4 μmole/kg of the chemical was gavaged in 7 g of pre-ingested food or in 50 μl of alcohol. The pre-ingested food, which the author called "ingesta," was obtained from the stomachs of rats that had engorged with food after a fast. The author did not specify the method used to mix the carbaryl with the ingesta.

Gavage in 50 μl of alcohol resulted in a peak blood level of carbaryl one minute after dosing and a subsequent decrease to roughly 25 percent of the peak level after 20 minutes. Gavage of the same dose of carbaryl in 7 g of ingesta resulted in a blood level at one minute that was less than 10 percent of the alcohol value. Two minutes after the ingesta gavage, a blood level less than 20 percent of the alcohol value was observed, and blood level remained at this value for 10 minutes, followed by a slight decrease at 20 minutes. A lipid-insoluble metabolite, which the authors presumed to be the glucuronide, appeared in both experiments at blood levels equal to or exceeding the carbaryl concentration.

The disappearance of carbaryl from the GI tract was also measured directly after gavage with the two different vehicles by analyzing the contents of the GI tract. The disappearance of carbaryl after alcoholic gavage was stated to be "essentially instantaneous and complete" but no data were shown for this experiment. The time course of the content of the GI tract after ingesta gavage showed a half-life of approximately 2.5 hours. At 6 hours, less than 5 percent of the dose of carbaryl remained in the GI tract. Approximately 20 percent of the dose appeared as a lipid-soluble metabolite in the GI tract at 1 hour after dosing. This component increased to about 25 percent at 6 hours and then decreased to less than 10 percent after 15 hours. It is not clear whether the metabolism of carbaryl to this compound occurred in the lumen of the GI tract prior to absorption or if it occurred following absorption with subsequent secretion of the metabolite into the GI tract. Unfortunately, the author did not state whether or not this metabolite appeared in the GI tract after alcohol gavage. This information would provide a strong indication of whether or not the metabolism occurred in the GI tract.

The rats in this experiment were fasted except for one animal which was allowed to consume a large quantity of food immediately after dosing. This rat was sacrificed at 4 hours, and the contents of its GI tract were measured. Approximately 80 percent of the dose was still present in the GI tract as carbaryl at 4 hours, with the remaining 20 percent present as a lipid-insoluble metabolite. This result dramatically confirms the effect of ingested food in slowing the absorption of a trace of nonnutrient chemical. The presence of an approximately equivalent amount of the metabolite in the GI tract in this animal which experienced virtually no absorption, compared to the fasted animals which had absorbed the majority of the dose by this time, suggests that the metabolism occurred in the GI tract.

The study of Pekas (1974) demonstrates a dramatic difference in GI absorption and metabolism upon ingestion of a chemical with a large volume of food compared to gavage in a small volume of alcohol, i.e., instantaneous absorption from alcohol vehicle versus absorption over the course of hours when ingesta accompanied the dose of carbaryl. This has major implications for the modeling of GI absorption. A dose of a chemical administered by oral gavage in a solvent may produce blood levels that are several fold higher than the same oral dose ingested as part of the normal diet. In addition, the delay of absorption by food will possibly change the site of absorption. The instantaneous absorption from a small volume of solvent after intragastric gavage would occur in the stomach. Delayed absorption accompanying food ingestion would more likely occur in the small intestine, as the nonnutrient material followed the transit of food through the GI tract. This could also affect the distribution of the chemical to tissues following absorption.

4.3 OSMOLARITY OF VEHICLE

The GI epithelium is permeable to the relatively rapid flow of water in both directions. Water flow occurs in response to osmotic gradients, as described in the appendix, to maintain the isotonicity of the luminal contents. Depending on the direction, osmotically-induced flow could either enhance or inhibit the absorption of compounds from the GI lumen. Ochsenfahrt and Winne (1974) demonstrated that the absorption rate of benzoic acid by isolated rat jejunal segments was linearly dependent upon the net water flux across the membrane as influenced by hypertonic, hypotonic, or isotonic solutions. An effect of the osmolarity of water vehicle on the apparent rate of GI absorption

in rats of both rapidly absorbed lipid-soluble compounds, and more slowly absorbed water-soluble compounds, was reported by Vogel et al. (1975). These authors instilled four different compounds through a jejunal cannula. Three different vehicles were tested for each compound. The vehicles were: isotonic saline, two times isotonic mannitol solution, and three times isotonic mannitol solution. Mannitol does not permeate the intestinal wall, and its presence in the lumen as a hyperosmolar solution results in osmotic flow of water from blood to lumen. Two slowly absorbed drugs, atropine and azoniaspiro compound XVII, and two more rapidly absorbed, lipid-soluble compounds, phenobarbital and nicotine, were studied. The rate of absorption of these compounds in each vehicle was determined indirectly. For phenobarbital, the time between instillation and unconsciousness (latent period) was used as an indirect measure of absorption. Percent mortality was the indicator used for absorption of the other three compounds. The dose of the compounds tested for mortality was near the LD_{50} value. Three out of the four compounds tested showed decreasing apparent rates of absorption with increasing osmolarity of the vehicle. The azoniaspiro compound XVII was the only one of the four compounds that did not exhibit an apparent decrease in absorption with increasing osmolarity of the dosing vehicle.

The magnitude of the effect of hyperosmolarity on the rate of absorption in this study cannot be estimated from the indirect determinations that were based on mortality. The percent mortality was 54 and 40 percent less for atropine and nicotine, respectively, when the dosing vehicle was 3 times isotonic mannitol compared with isotonic saline. This suggests that the blood or tissue concentrations resulting from the mannitol experiment were significantly less than with isotonic saline, but the difference cannot be quantitated from the data available. The latent period to unconsciousness from phenobarbital was 21.9 minutes with the three times isotonic mannitol solution compared to 12.7 minutes with isotonic saline. These data are somewhat more directly related to rate, and the difference is consistent with a 40 percent slower rate of absorption from the hyperosmotic vehicle.

The effect of osmolarity on the apparent rate of absorption in these experiments could be explained by at least two mechanisms. One mechanism would be the predominant flow of water in the direction opposite to uptake directly opposing the movement of material out of the lumen. A second would be dilution of the concentration of drug by the flow of water into the lumen with an

effect on the rate of diffusion of drug out of the lumen resulting from the decrease in concentration gradient. The authors speculate that both mechanisms may be in operation. Welling (1977), however, cited a number of studies that showed more rapid absorption associated with administration of more dilute solutions of compounds in water.

The effect of hypoosmotic vehicle on the GI absorption in humans was studied by Williams and Maddocks (1975). The absorption of phenol red, a strong acid that is lipid-insoluble and therefore slowly absorbed, and sodium salicylate, a compound that is more lipid-soluble and readily absorbed in the stomach, was studied with isotonic saline and water as the two dosing vehicles. The experiments were designed to test if the osmotic flow of water out of the GI lumen when water was the vehicle would increase the uptake of nonnutrient compounds from the lumen relative to the uptake in the presence of isotonic saline, which would not induce osmotic flow. Salicylate uptake was measured as the plasma concentration and the uptake of phenol red was quantitated as the urinary excretion. There was no difference between the absorption of salicylate with either water or isotonic saline as the vehicle. The authors concluded that this result was consistent with diffusion of this compound through the lipid phase of the membrane. Phenol red, in contrast, was excreted in urine in an amount that was nearly two-fold higher when it was administered with water compared to isotonic saline. This is consistent with an effect of "solvent drag," i.e., as the water rushes out of the lumen in response to osmotic pressure, water-soluble compounds are carried along, enhancing their rate of uptake. An alternative to this mechanism would be a concentration effect as the water vehicle left the lumen and the concentration of the compounds increased as the volume decreased. The authors cited the lack of vehicle effect on salicylate as evidence that concentration change is not the mechanism of the effect.

The lack of effect of osmotic flow out of the GI tract on the absorption of lipid-soluble salicylate in humans is in apparent contrast to the effect of osmotic flow into the GI tract of rats on the uptake of phenobarbital and nicotine in the study by Vogel et al. (1975). A likely explanation for this apparent inconsistency is that the compounds studied by Vogel et al. were deposited through a jejunal cannula, bypassing the stomach, so that absorption was in the intestine. Thus the results of Vogel et al. show an osmotic effect on absorption of both lipid-soluble and water-soluble compounds in the jejunum in rats, while the results of Williams and Maddocks show no effect of osmolarity on the

relatively rapid absorption of salicylate in the stomach of humans. The effect of osmolarity on the absorption of phenol red in the human study may have been observed because the major part of absorption of this more slowly absorbed compound occurred in the intestine.

4.4 SUMMARY

The vehicle in which a nonnutrient compound is dissolved or suspended can have a marked effect on the rate of absorption. Oil persists for hours in the GI tract and, when used as the vehicle, produces relatively slow and irregular absorption of lipid-soluble compounds. Water is rapidly absorbed from the GI tract and essentially deposits dissolved compounds in a concentrated form on the GI epithelium, resulting in more rapid absorption. In contrast to the effect of oil on lipid-soluble compounds, the absorption of poorly-soluble griseofulvin, and water-soluble heparin were enhanced by an oil in water emulsion compared to administration in water alone.

When associated with a large portion of food, a lipid-soluble compound is absorbed over the course of hours, as compared to instantaneous absorption when dosed in a few µl of alcohol. This probably reflects the partitioning of the compounds between the luminal contents and the GI epithelium. Food has less of an impact on rate of absorption of relatively insoluble compounds such as antibiotics. Osmotic gradients have been shown to have effects on absorption consistent with the osmotically-induced flow of water in the intestine, but not in the stomach.

The effect of vehicle on the uptake of trace nonnutrients from the GI tract can have a major impact on the pharmacokinetic disposition of the nonnutrients. Slower uptake from the GI tract in response to vehicle differences has been demonstrated to result in maximum blood concentrations that were an order of magnitude lower than those resulting from more rapid absorption. Also, the site of absorption can depend on whether the compound is deposited in an empty stomach, in which case a lipid-soluble compound will be quickly absorbed in the stomach, or is associated with food, which limits its contact with the GI epithelium and allows considerable passage along the GI tract before complete absorption occurs. In addition, the opportunity for metabolism in the GI tract is greatly enhanced by a vehicle that slows the absorption of the chemical.

SECTION 5

CRITICAL PARAMETERS IN THE MODELING OF ABSORPTION

This section discusses those critical parameters that require consideration in any attempt to define and model the events involved in GI absorption of nonnutrients and to extrapolate the results of these efforts between species or routes of exposure. With the exception of the properties of the substance itself, variations in these parameters between different organs of the GI tract or between species will potentially affect sites and rates of absorption and the distribution of the absorbed material.

5.1 TRANSIT TIME AND MOTILITY

The kinetics of absorption of materials from the GI tract is complicated by the migration of materials through the various organs of the GI tract. The time of occupancy for the various organs of the GI tract varies from a few seconds in the esophagus to days in the intestines. The stomach and small intestine are the major sites of absorption, the rate of which can vary significantly in the two different organs. The emptying of materials from stomach into intestine is, therefore, a critical event in the kinetics of absorption. The timing of gastric emptying can be significantly affected by the composition of the ingested material, and this is a parameter that must be known in any attempt to describe the kinetics of GI absorption in terms of physiological events.

The motility of material through the intestine is a parameter that determines the time of residence in the intestinal lumen. Intestinal motility, like gastric emptying, is affected by the composition of ingested material, and the resulting variations in transit time may significantly affect the kinetics of absorption. For compounds that are absorbed relatively slowly, the time of residence in the gut may affect the extent of absorption. In addition, since motility is a function of undulations and perturbations of the epithelial surface, the nature of the barrier and especially the unstirred layer of water, are affected by motility changes, which in this way could affect the process of absorption.

5.2 GI CONTENTS

The nature and composition of the GI contents must be considered in analyzing, comparing, or extrapolating GI absorption between experiments or species. Interaction between ingested compounds and the GI epithelium is critically dependent upon GI contents which affect solubility of the compounds of interest and also may produce significant physiological changes such as in flow of blood or lymph, bile secretion, gastric emptying, and motility.

5.3 SURFACE AREA

The amount of surface available for absorption is an important determinant of the capacity for absorption. The large area of the small intestine relative to other organs of the GI tract is a major factor in its function as the major site of absorption of nutrients and in its potential as a major site of absorption of nonnutrients. Comparison of the area of small intestine and other organs of the GI tract normalized to body weight or body surface area is important in comparing the absorption capacity between species. The human small intestine, for example, has about five times the surface area of the rat when normalized to the overall surface area of the body.

5.4 VASCULARITY: BLOOD AND LYMPHATICS

Since blood and lymph are the immediate destinations of materials absorbed from the GI tract, a thorough knowledge of the flow of blood and lymph through the GI tract is essential to a quantitative description of the absorption process. The origin, destination, and rates of flow are critical parameters for incorporating GI absorption into a complete quantitative toxicokinetic description of an ingested compound. Since blood and lymph vessels are the sites of entry into these circulating fluids, the anatomical arrangement and physical characteristics of the vessels that carry blood and lymph as it passes through the GI mucosa are critical factors to also be considered in describing GI absorption.

5.5 ENTEROHEPATIC CYCLING

The absorption of bile salts from the intestine, and their resecretion from liver to intestine, provides a potential mechanism which can significantly affect the kinetics of absorption of

nonnutrient compounds. Compounds that are absorbed from the GI tract into the portal circulation and excreted from the liver into the bile ducts to be secreted with bile back into the intestine will undergo a cycling process that could greatly complicate the kinetics of absorption. Enterohepatic cycling is subject to perturbation by effects of food on bile secretion. In addition, the absence of a gall bladder in the rat makes this parameter of particular importance in extrapolating rat data to other species. Large variance in enterohepatic cycling among species requires that this effect be carefully considered in comparing data between species.

5.6 CHEMICAL AND PHYSICAL PROPERTIES OF THE INGESTED COMPOUNDS

While the compound itself remains a constant in extrapolation between exposure routes or species, the chemical and physical nature of the compound determines which of the above parameters will be a factor in its absorption. Lipophilic compounds are absorbed relatively rapidly so that surface areas or transit times are not likely to be rate limiting in their absorption. The vehicle in which these compounds are administered, however, can have a significant effect on their absorption. Water-soluble compounds, which are absorbed more slowly, are more susceptible to effects of differences in motility or surface area.

The route of entry of absorbed materials into the circulatory system may also be affected by the chemical nature of the absorbed compound. Lipophilic compounds ingested with dietary lipid may associate, in the interstitial fluid, with the chylomicron particles formed from dietary lipid. As a result of association with the relatively large chylomicrons, movement of such compounds into blood capillaries would be limited by the size of the chylomicrons, and they would tend to accompany the chylomicrons into the lymphatic lacteals rather than the blood capillaries.

SECTION 6

IMPACT OF THE CRITICAL PARAMETERS ON THE ABSORPTION OF VOLATILE HALOGENATED HYDROCARBONS

This section discusses the results of evaluation of pharmacokinetic data available in the published literature for oral administration of volatile halogenated hydrocarbons containing one or two carbon atoms. The data were evaluated for evidence regarding the impact of the six critical parameters, defined in the previous section, on GI absorption of these compounds. The kind of data needed for this type of evaluation was found to be scarce. The available data demonstrated a significant impact of three of the six parameters on absorption of these compounds. Most of the data addressed the effects of GI contents. An important role of transit time, in particular gastric emptying, is also consistent with the data when the effect of GI contents on these parameters is considered. The fact that different chemicals of this class, which share common physical properties, showed similarities in the rate and extent of absorption and the effects of vehicle illustrates that the physical properties of chemicals are significant determinants of GI absorption.

6.1 THE NATURE OF THE DATA REFLECTING GI ABSORPTION

Three types of pharmacokinetic data were identified that yield information on rate or extent of GI absorption of volatile halogenated hydrocarbons. Perhaps the most direct measurement is the amount of administered chemical that remains in the GI tract. Angelo et al. (1986a, 1986b) reported the time course of dichloromethane content in the GI tract of mice (1986a) and rats (1986b) after oral administration. In the mouse study, water and oil were compared as vehicles, and the rate of absorption of dichloromethane from water was observed to be much faster than from oil, as described in section 4.

All of the remaining studies of these compounds reported either blood concentrations or concentrations in exhaled air as a function of time after dosing. Exhaled air concentrations have been shown to be proportional to blood concentrations for chloroform (Fry et al., 1972). This proportionality is likely to be true for all volatile compounds which move from pulmonary blood into alveolar air by simple diffusion. Thus these two measurements are similarly useful in studying GI

absorption. They are less direct measurements of GI absorption than measurements of the amount of chemical remaining in the GI tract itself, because they are influenced by several other events, most notably the uptake and release of chemical by other tissues.

After the oral administration of volatile halogenated hydrocarbons, the time courses of both the blood concentration and the concentration of these chemicals in exhaled air exhibit biphasic decreases (Putcha et al., 1986; Chieco et al., 1981; D'Souza et al., 1985; McKenna et al., 1978; Pegg et al., 1979). The initial decrease reflects loss of chemical from blood to tissues after an initial increase in blood concentration as the chemical is absorbed. In this initial phase, blood is functioning to distribute the chemical to tissues as it is absorbed from the GI tract. Concentration in blood generally exceeds tissue concentrations, and the net movement of chemical is out of the blood and into tissues, including the lung, where the chemical is exhaled and lost from the body in proportion to its concentration in blood. In the later phase, where loss of chemical from the blood is slower, the blood is functioning to eliminate the chemical from tissues, carrying it from tissues to lung, where it is exhaled. In the extent that the absorption of chemical from the gut continues through this later phase, the rate of decrease of chemical concentration in blood or expired air will be affected by uptake of chemical from GI tract to blood. An effect of an experimental variable on GI absorption during this later phase after oral administration may be detected as an effect on the concentration of chemical in blood or exhaled air. The data available suggest that volatile halogenated hydrocarbons are absorbed from the GI tract in two phases, an initial rapid phase and a slower long-term phase, and effects of GI contents on both phases are apparent.

6.2 EFFECT OF GI CONTENTS AND TRANSIT TIME

The contents of the GI tract, and the time that the chemical is in the different organs of the GI tract, are the parameters for which the available data shows the most evident impact on GI absorption of the volatile halogenated hydrocarbons. GI contents varied in the experiments reviewed either as a result of the use of different vehicles for the administration of the compounds, or as a result of whether the animals were fasted or fed. Significant differences in the rate of absorption with oral dosing in oil compared to aqueous vehicle, and differences between rates of absorption in fasted and fed animals are consistently shown for the various chemicals of this class.

The study by Withey et al. (1983) showed more rapid absorption of four halogenated hydrocarbons from water compared to oil, as described in section 4. This effect can be further confirmed by comparison of a number of studies of single chemicals administered in a single vehicle. Both dichloroethylene (Putcha et al., 1986) and trichloroethylene (D'Souza et al., 1985) showed peak blood levels within 10 minutes after dosing in 50 percent aqueous ethlene glycol. This is in contrast to experiments where dichloroethylene was administered in corn oil, in which peak concentrations in exhaled air were observed in 30 to 60 minutes (McKenna et al., 1978), and for tetrachloroethylene in corn oil, which yielded a peak blood concentration in 60 minutes (Pegg et al., 1979). Dosing of humans with chloroform in olive oil (Fry et al., 1972) also showed peak blood levels of this compound in 1 hour. Thus, a comparison of these studies shows that the time to peak blood levels was 3 to 6 times longer in the studies that used an oil vehicle compared to the studies that used an aqueous vehicle. This is consistent with the range of differences for time to peak blood levels reported in the study of Withey et al. (1983).

Chieco et al. (1981) compared the exhalation of dichloroethylene by rats orally dosed with three different vehicles. The vehicles used were mineral oil, which is neither digested nor absorbed, corn oil, which is digested prior to absorption, and water, which is rapidly absorbed, as described in section B.2.1. (The water vehicle contained 0.5 percent Tween 80.) Exhalation data were obtained at 15 minute intervals. There was no difference in the half-life of exhalation during the first hour for any of the three vehicles. The absence of any measured effect of vehicle on the initial absorption in this study, which is in seeming contrast to the effects described above, may be due to the fact that the first data point was not obtained until 15 minutes after dosing. For exhalation that occurred after hour two, however, the half-lives of elimination differed by a factor of 2 or 3 for the three different vehicles. The authors interpreted this to be an effect of vehicle on the rate of GI absorption. Mineral oil produced a long-term exhalation half-life that was about three times longer than that of corn oil. The authors attributed this difference to the fact that mineral oil is not digested or absorbed and thus remains in the gut as a solvent for the dichloroethylene, delaying absorption so that it occurs over a relatively extended time period. Continuing absorption from GI tract to blood over a long time period results in an apparently slower disappearance of the chemical from blood. Corn oil has a shorter residence time in the gut because it is digested and absorbed and therefore has a less prolonged effect

on absorption, allowing the dichloroethylene to be absorbed faster. Water containing Tween 80 as vehicle yielded a long-term half-life that was two or threefold shorter than corn oil. The authors suggested that this was due to the relative insolubility of dichloroethylene in the aqueous vehicle and possibly to the effect of the detergent Tween 80 on the mucosal barrier. Another explanation for the relatively rapid uptake of dichloroethylene from water is the rapid absorption of water from the GI tract as discussed in section 4.

The reported effect of food in the GI tract on GI absorption varies with different studies, but fed animals generally exhibited a slower uptake of chemical during the initial phase (D'Souza et al., 1985) or a longer half-time of elimination over the longer term than fasted animals (Putcha et al., 1986), which the authors interpreted as indicating delayed absorption. These effects of food on GI absorption are likely due to adsorption of a portion of the chemical to food components or dissolving of a portion of the chemicals in the lipid component of the food.

A study which appears to contradict to the generally observed effect of food in slowing absorption is that of McKenna et al. (1978), in which fasted rats appeared to absorb dichloroethylene more slowly than fed rats. Peak exhalation of dichloroethylene administered by gavage in corn oil occurred at 1 hour for fasted animals and at 30 minutes for fed rats. Fasted rats also exhaled more of the total dose of dichloroethylene unchanged than fed rats, consistent with prolonged absorption and elimination. The greater amount of dichloroethylene exhaled unchanged and the more prolonged exhalation of dichloroethylene in the fasted rats are both consistent, however, with expected effects of fasting on direct loss of volatile dichloroethylene from the GI tract into exhaled air by way of gastric eructation. Gavage of oil into an empty stomach might delay gastric emptying by the mechanism described in sections 2.4 and A.3.4 which would allow more time for direct loss of dichloroethylene from the stomach. An increased loss of unmetabolized dichloroethylene directly from the stomach would increase the percent of chemical that was exhaled unchanged. The presence of food in the stomach during gavage of the dichloroethylene dose in corn oil, in contrast, would dilute the oil solution of dichloroethylene, decreasing the release of dichloroethylene vapor in the stomach. Food in the stomach may also increase the rate of gastric emptying which would decrease the time during which dichloroethylene vapor could be lost directly from the stomach. This explanation could be

tested experimentally by measuring the separate contributions of lung and stomach to the "exhaled" dichloroethylene, as described in section 7.

6.3 PROPERTIES OF THE CHEMICAL

Volatile halogenated hydrocarbons are low molecular weight, highly diffusable, lipid-soluble compounds. These characteristics are consistent with rapid diffusion of these compounds through the lipid membranes of the GI epithelium and with the observed rapid absorption of these compounds. Rapid absorption and similar effects of vehicle and food on different compounds in this class are consistent with chemical properties as a critical parameter affecting the GI absorption of these compounds.

One of the differences that exists within this class of chemical is a difference in water solubility between dichloroethylene (0.25 g/100 ml) and trichloroethylene (0.1 g/100 ml). This difference correlates with a difference in the apparent completeness of GI absorption. Completeness of absorption is assessed by comparing the total area under the curve of a plot of blood concentration versus time for animals dosed orally with animals receiving the same dose intravenously. The ratio of these two measurements, expressed as a percentage, is also known as the bioavailability.

The bioavailability of dichloroethylene was determined by Putcha et al. (1986). They reported no significant differences in the area under the curve of the plot of blood concentration versus time for intravenously- and orally-dosed dichloroethylene in aqueous polyethylene glycol over a 10-fold range of dose (10 to 100 mg/kg) for fasted and fed rats. This indicates 100 percent bioavailability of dichloroethylene from the GI tract, independent of dose or feeding status. D'Souza et al. (1985) showed complete absorption of an oral dose of 10 mg/kg of trichloroethylene in aqueous polyethylene glycol from the GI tract of fasted rats by the same technique. In the same study, however, bioavailability of trichloroethylene was significantly less than complete in fed rats and decreased with increasing dose from 78 percent at 5 mg/kg to 58 percent at 25 mg/kg. The less than complete bioavailability of trichloroethylene in fed rats may be due to its lesser solubility in water which would result in a relatively greater partitioning into the lipid portion of GI contents of fed animals and a more limited tendency, in the presence of lumenal lipid, to traverse the aqueous layer that lines the GI epithelium.

SECTION 7

SUGGESTED EXPERIMENTAL APPROACHES TO EVALUATING THE IMPACT OF PARAMETERS ON GI ABSORPTION

It is evident from the last section that there is a scarcity of data in the published literature that conclusively demonstrates the impact of the identified individual critical parameters on GI absorption of the volatile halogenated hydrocarbons. This section describes, in general terms, some experimental approaches to further defining the role of the critical parameters in GI absorption of these compounds.

7.1 OPTIMAL EXPERIMENTAL DESIGN

Early measurement of the chemical concentration in tissues is critical in characterizing GI uptake in the absence of confounding effects. The time of peak blood concentration was the most useful parameter available in the data analyzed, and for the volatile halogenated hydrocarbons which are relatively rapidly absorbed, the time to peak concentration in blood is a crude indicator of initial rate of absorption. The available data suggest, however, that the total dose is not absorbed homogeneously during the initial uptake and the data are consistent with continuing absorption of a portion of orally administered volatile halogenated hydrocarbons during a slower, long-term phase. The physical basis for the biphasic absorption of these chemicals is not known. Further experimental work is necessary to define the two phases and to provide a possible physical explanation for them. The ideal experiment would obtain several measurements of blood concentrations and GI content of the administered chemical at early time points, prior to the peak blood concentration, in addition to longer term measurements. Early data on the amount of chemical in the stomach and different regions of intestine, together with blood concentrations, would allow a quantitative description of the two phases of absorption. Using this experimental design and comparing the effects of oil and water as vehicles, and the results in fasted and fed animals, may provide data which can be used to define more quantitatively the role of the critical parameters in the two phases of absorption.

7.2 GI CONTENTS AND TRANSIT TIME

The available data provided a consistent qualitative picture of the effects of oil versus water, and fasting versus feeding, on the absorption of the volatile halogenated hydrocarbons. A more

systematic experimental approach, as described above, would be necessary to quantitatively define the GI absorption process for a specific chemical and specific experimental conditions. The only data analyzed that is inconsistent with a retarding effect of food or oil on absorption is that of McKenna et al. (1978), in which exhalation data were presented. As discussed previously, the results of McKenna et al. are consistent with direct loss of a portion of the volatile chemical from the stomach by gastric eructation. The role of direct loss of volatile chemicals from the stomach could be assessed by separate cannulations of the esophagus and trachea after oral dosing.

7.3 PROPERTIES OF THE CHEMICAL

Comparison of the results of Putcha et al. (1986) for dichloroethylene with those of D'Souza et al. (1985) for trichloroethylene suggested an effect of solubilities on GI absorption, as discussed previously. The water solubilities of tetrachloroethylene (0.048 g/100 ml) and trichloroethylene (0.100 g/100 ml) are similar and both considerably less than the solubility of 1,1-dichloroethylene (0.25 g/100 ml) or dichloromethane (1.96 g/100 ml). These four chemicals could be compared experimentally to test the effect of water solubility on GI absorption. Oral administration of these chemicals in oil and water to fasted and fed animals, coupled with measurements of blood concentrations and GI content of chemicals at frequent early time points, as well as over a more extended time period, should provide a better understanding of the effect of solubility differences on the absorption of this class of compound.

7.4 VASCULARITY

There is no information in the data analyzed to indicate the distribution of volatile halogenated hydrocarbons absorbed from the GI tract between blood and lymph. The lipid-soluble nature of these compounds, however, raises the possibility that they would partition to some extent into the droplets of dietary lipid (chylomicrons) that are taken up by lymph. Since the volume of lymph flow and the uptake of chylomicrons are both affected by the presence of food or lipid in the GI tract, and since blood and lymph follow very different routes of distribution to the rest of the organism, the effects of vehicle and feeding state on blood concentrations of chemical could be partially a result of effects on lymph flow and the availability of chylomicrons moving from the GI tract into the lymph. In the fasted state, lymph flow in the GI tract would be relatively small, and the lymph would contain no

chylomicrons. In fed animals, or with administration of chemical in a digestible oil, lymph flow would be stimulated, and the presence of chylomicrons being absorbed from GI tract into lymph would favor partitioning of the volatile halogenated hydrocarbons into lymph.

The role of blood versus lymph in the uptake of volatile halogenated hydrocarbons from the GI tract could be experimentally examined by cannulation of the hepatic portal vein, for sampling of blood, and the cysterna chyli, for sampling of lymph, and measuring the concentration of the chemical in these two fluids. Samples should be taken as soon as possible after dosing so that the initial uptake can be characterized. The amount of chemical expected in lymph could be maximized by performing the experiment first in fed rats administered the chemical in an oil vehicle. If significant amounts of the chemical were found in lymph under these conditions, the partitioning of the chemical between blood and lymph should be quantitated for other conditions as well. This experiment should be performed at doses where the expected concentrations of chemical in the blood would be both above and below the aqueous solubility. As the solubility limits of the chemicals in water are exceeded, the partitioning of the chemical into chylomicrons would be expected to be favored. The partitioning of chemical into chylomicrons versus water above and below the limits of solubility could also be determined *in vitro* with samples of lymph exposed to chemical.

7.5 ENTEROHEPATIC CYCLING

There is no direct evidence that volatile halogenated hydrocarbons undergo enterohepatic cycling, and their chemical structure and properties are different from the type of chemicals that are known to undergo enterohepatic cycling. Biphasic GI absorption is characteristic of chemicals that undergo enterohepatic cycling, however, and the effect of oil in slowing the appearance of these chemicals in blood could theoretically be partially a result of an increased flow of bile if the chemicals were re-entering the GI tract with secreted bile. Measuring the concentration of orally administered volatile halogenated hydrocarbons in bile would allow an estimation of the extent to which these chemicals undergo enterohepatic cycling. It is important that the collection of bile for such measurements does not disrupt the normal flow of bile. A sample of bile should be obtained for analysis without total diversion of the bile flow from liver to GI tract for any significant period of

time. Diversion of bile flow would change any normal effect of bile on absorption of the chemical as well as the return of chemical to the GI tract via bile from the liver.

7.6 SURFACE AREA

Because the volatile halogenated hydrocarbons are absorbed relatively rapidly from the GI tract, it is unlikely that the surface area of the tract will be rate limiting. Therefore, experiments addressing the role of this parameter on the absorption of these compounds are not likely to be necessary in a quantitative description of the GI absorption of these chemicals.

SECTION 8

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APPENDIX A

COMPARATIVE PHYSIOLOGY AND ANATOMY OF THE GASTROINTESTINAL TRACT

This appendix systematically describes the organ systems of the human GI tract, with emphasis on features relevant to absorption. Corresponding characteristics in laboratory animals will be presented where information is available.

A.1 MOUTH

A.1.1 General Description and Function

The mouth is the entry to the alimentary canal. It is the site of the beginning of the digestive process. The functions of the mouth include chewing (mastication), salivation, and swallowing (deglutition). The mouth has a complex structure that includes diverse integral structures such as teeth, the tongue, and the uvula, in addition to important adnexal structures including the salivary glands.

A.1.2 Nutrient Processing

The mouth is the site of the initial processing of orally ingested materials. The processing consists of the physical breaking down of the ingested material through chewing, the addition of saliva to the ingested bolus, the mixing of saliva with the ingested material (another result of chewing), and the beginning of digestion of carbohydrates. Much of the beginning of digestion is due to the action on the ingested food by enzymes contained in the saliva. The most important enzyme secreted by the salivary glands is α-amylase (ptaylin) which hydrolyzes the α-1,4 glycosidic bonds of polysaccharide molecules such as are found in starch. This enzyme is sensitive to changes in pH and is rapidly inactivated by the acid conditions in the stomach. Another important enzyme is lingual lipase which is capable of digesting fats. In contrast to α-amylase, lingual lipase is not inactivated by acid conditions and remains active in the stomach.

The action of saliva on ingested food is enhanced by the mastication of the bolus by the teeth and tongue. Mastication breaks the food into small particles that have a greater surface area for

interaction of the food with salivary enzymes and, eventually, with gastric juice. The extent of carbohydrate digestion that takes place in the mouth is controlled by the thoroughness and amount of time that is spent in chewing.

A.1.3 Epithelial Characteristics

The epithelium of the oral cavity is of the moist stratified squamous type. In some regions, such as the dorsum of the tongue and the hard palate, the epithelium is keratinized. Epithelia of these types are not found in areas of the body that play a major role in absorption. Rather they are found in areas that are subject to physical stress. Due to the trauma that occurs during mastication, it is not surprising that a non-absorptive type of epithelium is found in the mouth.

A.1.4 Secretory Function

A prominent activity of the mouth is secretion of saliva. Saliva is a complex secretion of three pairs of major salivary glands (parotid, submandibular, and sublingual) and numerous minor salivary glands located throughout the oral cavity. Saliva is a watery secretion that is hypotonic relative to plasma with a pH of approximately 7.0 (normal range from 6.0 to 7.0). A saturating concentration of calcium in saliva prevents loss of dental calcium (Ganong, 1979). Saliva contains several important proteins including the digestive enzymes α-amylase and lingual lipase as well as mucin and epidermal growth factor. As mentioned in section A.1.2, the digestive enzymes initiate the process of digestion. The mucin acts to coat the food bolus thereby promoting the ready passage of food into the alimentary canal and protecting the luminal epithelium. Epidermal growth factor is an important stimulus to growth of the luminal epithelium of the stomach (Granger et al., 1985).

In humans, the daily volume of salivary secretion is estimated to be 1,200 ml (range 1,000 to 1,500 ml; Guyton, 1971). The percentage of daily secretion by each of the major salivary glands are parotid glands 20 to 25 percent, submandibular glands 60 to 70 percent, and sublingual glands 5 to 20 percent (Snyder et al., 1975; Ganong, 1979). During periods of maximal secretion, the salivary glands of humans can secrete their own weight in saliva every ten minutes whereas the salivary glands of the dog can secrete their own weight in only two minutes (Granger et al., 1985). To gain an appreciation of the magnitude of this secretory rate, it should be noted that in some animals the

submandibular salivary gland can secrete saliva at a maximal rate of 1 ml/gram of tissue/minute compared to the maximal rate of the entire pancreas, which is 1 ml/minute (Jacobson, 1985).

A.1.5 Absorption of Nutrients

The primary functions of the mouth are the mastication of food and the secretion of saliva as initial steps in the process of digestion. Neither the anatomy of the oral epithelium nor the relatively short duration of time that food is in the mouth are conducive to the absorption of nutrients.

A.1.6 Bacterial Flora

While the mouth possesses a rich and diversified microfloral population in all species, the bacteria at this site do not have an appreciable effect on absorption. This is due to the relatively short time that most orally ingested material is in contact with bacteria that are resident in the mouth. Residual ingested material that remains in the mouth after swallowing is acted upon by bacteria. The results of this bacterial metabolism is the production of an acidic environment which is buffered to a large degree by the salivary content of bicarbonate ion. While the results of bacterial metabolism in the oral cavity have been associated with the development of dental caries, they have not been shown to be important in the absorption of materials in the mouth. Consequently, the oral bacterial flora will not receive further treatment in this document.

A.1.7 Adventitious Absorption

Although the mouth does not exhibit those anatomical characteristics that are associated with those portions of the alimentary canal that are proficient in absorption, a small amount of absorption of some substances can occur there. The region of the mouth that is responsible for the adventitious absorption of substances is the sublingual region: the underside of the tongue and the floor of the mouth immediately beneath the tongue. This region is more conducive to absorption in the mouth because the stratified squamous epithelium is particularly thin and the submucosa, which is absent on the other regions of the tongue, contains a relatively rich blood supply. The ability of amphipathic compounds with moderate lipophilicity to be absorbed in this region has been exploited for the organic nitrate compounds, such as nitroglycerin and isosorbide dinitrate, that are used in the treatment of acute angina pectoris (Gilman et al., 1980). These compounds are routinely prescribed to

be administered by the sublingual route. It is probable that at least a portion of any substance that exhibits similar chemical characteristics will be absorbed by this route provided that the substance is in contact with the sublingual epithelium for a sufficient period of time.

A.1.8 Dimensions and Transit Time

The dimensions of the mouth in the adult human male are 70 to 75 mm (antero-posterior) by 40 to 45 mm (transverse) by 20 to 25 mm (vertical) (Snyder et al., 1975). If the mouth is assumed to be a rectangle, its gross volume can be calculated to be 56 to 84 cm^3. Transit time in the mouth is a function of the amount of time that is required to prepare the ingested material for passage through the alimentary canal. For most liquids, such preparation is unnecessary; transit time of liquids in the oral cavity is relatively short and may be only the time necessary to perform deglutition (1 to 2 seconds) (Granger et al., 1985). For solid foods, the extent of time spent in mastication is the determining factor; for dry foods, such as crackers, this may be as long as 30 seconds or more.

A.1.9 Blood Flow

The arterial supply to the mouth, tongue, and salivary glands is by way of various branches of the external carotid artery. The venous drainage is by way of tributaries of the internal jugular vein and, for the parotid gland, the external jugular vein. Both of these veins eventually drain into the superior vena cava and thence to the right atrium of the heart. The importance of this route of return to the heart is that any substance that is absorbed in the mouth and distributed by the blood vascular system will pass to the heart without first traversing the liver. The first capillary bed to be encountered by these substances is located in the lung.

The vascular perfusion rates for the buccal mucosa and the tongue were not available. The resting blood perfusion rates for the salivary glands in humans is 50 ml/min/100 g of tissue (Granger et al., 1985). While this perfusion rate is similar to that of other gastrointestinal organs, the vascular perfusion can increase to 1,000 percent of the resting rate during periods of maximal stimulation, whereas other gastrointestinal organs exhibit increases of perfusion rates of only 30 to 130 percent (Granger et al., 1985).

To date, perfusion rates for rats and other laboratory animals have not been identified. However, the blood content of the submandibular salivary gland in rats is reported to be 81 µl/g of tissue and 110 µl/g of tissue in mice (Snyder et al., 1975). Data regarding the blood content of salivary glands in humans has not been found.

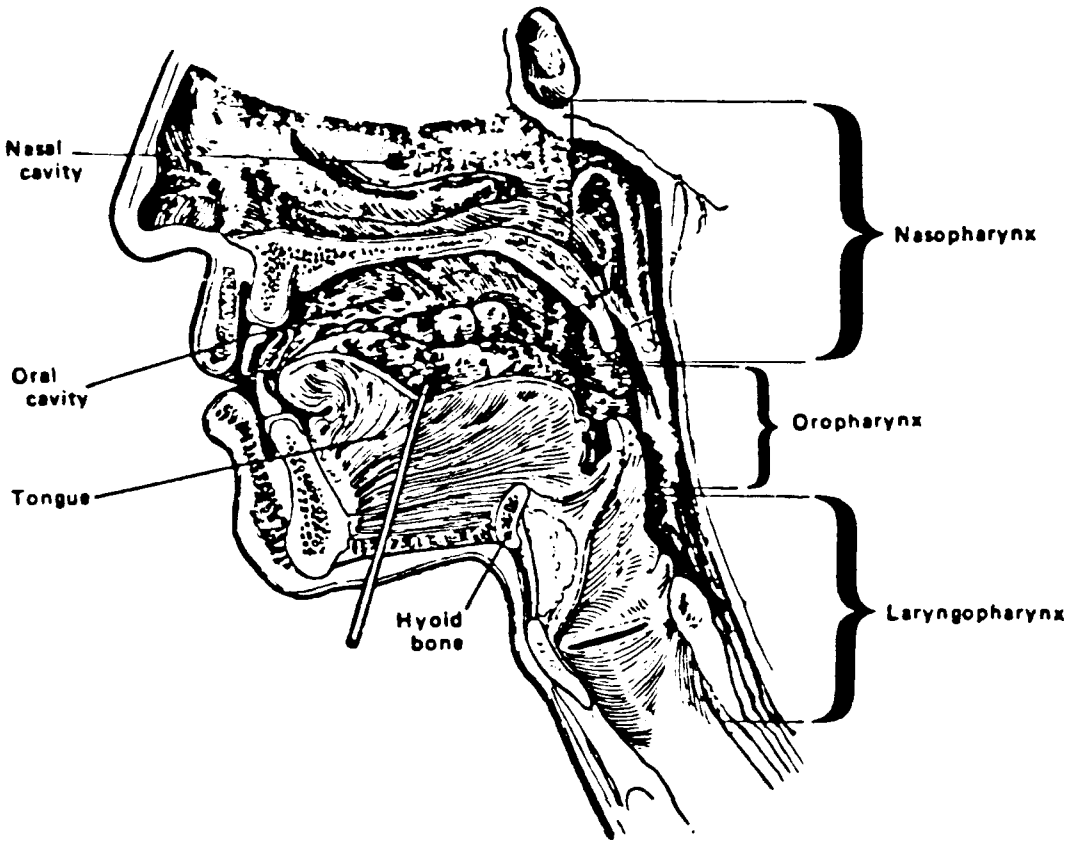
A.1.10 Lymphatic Flow

The richest arrangement of lymphatic vessels in the mouth occurs in the tongue and in the floor of the mouth at the underside of the tongue. This territory is also the most important for the adventitious absorption of substances. The lymphatic vessels of the tongue and floor of the mouth exhibit no special arrangement that corresponds to the lacteals of the small intestine. The lymphatic vessels do exhibit some complexity in their distribution in that they cross the midline to drain into nodes on the contralateral side of the body. These lymphatic vessels ultimately join the thoracic duct on the left side of the body or the right lymphatic duct on the right side. Both of these vessels join the venous system in the root of the neck. Substances that are transported by the lymphatic vessels would therefore also bypass the liver.

A.2 PHARYNX AND ESOPHAGUS

A.2.1 General Description and Function

The pharynx and esophagus are hollow muscular structures that serve as tandem conduits for the transfer of ingested material from the mouth to the stomach. The pharynx is shaped like a cone that serves as a common passageway for respiratory and digestive systems. The pharynx is continuous anteriorly with the mouth and nasal cavity and inferiorly with the esophagus and larynx. The pharynx is subdivided into thirds as depicted in figure A-1. The nasopharynx is located behind the nasal cavity and is continuous inferiorly with the oropharynx. The nasopharynx is not involved with ingestion or swallowing, and it is omitted from the remainder of the discussion. As used herein, the pharynx will refer to the combined oro- and laryngopharynges. The oropharynx is located directly posterior to the mouth; it is continuous with the nasopharynx superiorly and the laryngopharynx inferiorly. The laryngopharynx is the entryway to the esophagus for the alimentary canal and to the larynx for the respiratory system.



Source: Adapted from Longley et al., 1974.

Figure A-1
Subdivisions of the Pharynx

The esophagus is a cylindrical tube that extends through the thorax and connects the pharynx, located in the head, with the stomach, located in the abdominal cavity. In cross section, the lumen of the esophagus is seen to be invaginated by longitudinal folds of esophageal mucosa. These modifications of the luminal surface epithelium are for the purpose of providing a ready store of mucosa to accommodate rapid expansion of the lumen that is required when a bolus of food is passed through the esophagus. The folds are not modifications that increase the absorptive surface area of the mucosa as is true in the small intestine. Since the primary purpose of these organs is the expedient transfer of material from the orifice of ingestion (the mouth) to the first of the true digestive organs (the stomach), it is not surprising that these parts of the GI tract play essentially no role in the absorption of ingested material.

A.2.2 Nutrient Processing

The only nutrient processing to occur in the pharynx or esophagus is the continuing action of digestive enzymes secreted in saliva which accompany the food as it is transmitted to the stomach.

A.2.3 Epithelial Characteristics

The epithelium of the pharynx and esophagus is of the moist stratified squamous type. This type of epithelium is not well suited for absorption of materials; rather, it is found in areas of the body that must endure some physical stress. Due to the physical stress and trauma associated with swallowing, it is not surprising that this type of nonabsorbing epithelium is found in the pharynx and esophagus. In humans, the average cell life span for epithelial cells of the esophagus is estimated to be 8 days, and the entire epithelium is renewed approximately every 18 days (Iatropoulos, 1986). In rats, the cell life span is approximately 80 hours, and the epithelium is renewed every 7 to 8 days (Iatropoulos, 1986).

A.2.4 Secretory Function

Neither the pharynx nor the esophagus produce digestive secretions. In the pharynx, some minor salivary glands are present; in the esophagus, mucus glands are present, especially in the upper third. The function of both of these types of glands is to aid in the lubrication of ingested material for ready passage into the lower alimentary canal.

A.2.5 Absorption of Nutrients

The primary function of both the pharynx and the esophagus is the transport of ingested materials from the oral cavity to the stomach. Neither the anatomy of the epithelia nor the extremely short duration of time that materials are in contact with these epithelia are conducive to the absorption of nutrients. Under normal conditions, essentially no absorption occurs in these organs.

A.2.6 Bacterial Flora

The pharynx possesses a population of microflora that is similar to that of the mouth; as in the case of the mouth, the bacteria at this site do not have an appreciable effect on absorption. This is due to the very short time that orally ingested material remains in the pharynx.

The bacterial flora of the esophagus are greatly reduced in numbers when compared to the flora in the mouth. This is accounted for by the swallowing that occurs even during the resting (nonfeeding) periods of the day; the swallowing serves to reduce the bacterial population by washing bacteria into the stomach. As in the case of the mouth and pharynx, the bacteria in the esophagus play no appreciable role in absorption of orally ingested materials.

A.2.7 Adventitious Absorption

As stated above, neither the epithelial characteristics nor the duration of contact between ingested materials and the mucosae of the pharynx and esophagus are conducive to the absorption of materials. For virtually all normal circumstances, these two organs can be assumed to play no effective role in the absorption of ingested materials into the body. In some circumstances, delayed esophageal emptying may occur as the result of nervous system disorders (neuroses) or intrinsic absence of the autonomic innervation of the esophagus (Shepard, 1971). Under these relatively rare circumstances, adventitious absorption of materials may occur near the inferior end of the esophagus. The materials that are likely to be absorbed under these relatively rare circumstances are non-ionized, amphipathic substances with moderate lipophilicity.

A.2.8 Dimensions and Transit Time

The entire pharynx of the adult human male is approximately 15 cm in length; the surface area of the walls has been calculated to be approximately 300 mm^2 (Snyder et al., 1975). The surface area is smaller than expected due to the fact that much of the potential area of the pharyngeal walls is occupied by openings into other cavities (including the oral and nasal cavities, the openings of the auditory tubes; see figure A-1) and by the lymphoid tissue of the tonsillar ring (palatine, lingual, and pharyngeal tonsils). The dimensions of each of the three regions follow. The length is measured from superior to inferior; the cross sectional diameters are described as ovals. The nasopharynx is 3 cm long and has antero-posterior by transverse diameters of 2 by 4 cm; the oropharynx is 5 cm long with diameters of 4 by 5 cm; and the laryngopharynx is 7 cm long with diameters of 2 to 3 by 2 cm (Snyder et al., 1975). The transit time for a masticated bolus of food through the pharynx is estimated to be 1 to 2 seconds (Granger et al., 1985).

In humans, the esophagus of the adult male is 25 cm in length and weighs 40 g (Snyder et al., 1975). Due to the tonus of the circular layer of muscle, the lumen of the esophagus is thrown into numerous folds. These folds are distended during the passage of a food bolus. The diameter of the esophagus is 16 to 22 mm except in the regions of physiological constrictions where the diameter is 13 to 19 mm (Snyder et al., 1975). The transit time for a bolus of food to traverse the esophagus is approximately 6 seconds (Granger et al., 1985).

A.2.9 Blood Flow

The arterial supply to the pharynx is by way of various branches of the external carotid artery. Venous drainage occurs superiorly to the pterygoid plexus and inferiorly to the internal jugular vein which is joined by the pterygoid vein prior to joining the superior vena cava. Due to the virtual absence of absorption of materials by the pharynx, the importance of this route of return to the heart is minimal.

The arterial supply to the esophagus is derived from several sources. The cephalic esophagus receives vascular supply from the inferior thyroid artery of the thyrocervical trunk. The mid portion of the esophagus receives vascular supply from branches of the bronchial arteries and small branches

directly from the aorta. The inferior portion receives supply from the left gastric artery and the inferior phrenic artery. Venous return from the cephalic portion of the esophagus is via the inferior thyroid vein to the superior vena cava; from the mid portion blood returns via the azygos system of veins ultimately to the superior vena cava. The inferior portion of the esophagus has two potential routes of venous drainage. Blood may return via the phrenic vein and other small branches to the azygos system or it may follow the gastric veins ultimately to the hepatic portal vein and thence to the liver. Thus, the inferior esophagus is the site of an anastomosis between the portal and caval venous systems. If materials were absorbed in the inferior esophagus, some of the absorbed material could bypass the liver. This can be of importance not only when delayed esophageal emptying is present but also when portal hypertension is present (as in cases of hepatic cirrhosis or liver tumors). Under the latter conditions, blood from the gastrointestinal tract that would normally traverse the liver en route to the heart is forced retrograde from the liver back through the portal vein to the splenic and ultimately to the gastric veins and into the azygos system, thereby causing enlargement of the vein in the walls of the esophagus (esophageal varices) and allowing a significant portion of the absorbed material to bypass the liver. Although such bypassing of the liver is not commonplace during the healthy state, it can occur among alcoholic humans and among laboratory animals treated with liver toxicants.

A.2.10 Lymphatic Flow

The lymphatic drainage of the pharynx is to the retropharyngeal and upper deep cervical nodes and thence, ultimately, to the thoracic duct on the left side of the body or to the right lymphatic duct on the right side of the body. The probability that this route of absorption will affect the absorption of ingested material is virtually nonexistent.

The lymphatic drainage of the esophagus is primarily by way of the thoracic duct. This route of return bypasses the liver, however, as described above in section A.2.9, this route will only be important during certain pathological conditions. The lymphatic route would be expected to be most important during cases of delayed esophageal emptying; it is not expected to be of any consequence in cases of portal hypertension.

A.3 STOMACH

A.3.1 General Description and Function

The stomach is a hollow muscular organ in which the digestion of food begins in earnest. Labelled diagrams of important regions of the human and rat stomachs are depicted in figures A-2 and A-3. Masticated food is received from the esophagus in the region of the cardia, the food is retained in the large central portion of the stomach called the corpus where the initiation of digestion begins, and partially digested chyme is released to the duodenum via the pylorus. The pylorus is a muscular ring that closes off the gastric lumen from the lumen of the first part of the small intestine. The portion of the stomach that leads from the corpus to the pylorus is termed the antrum. The functions of the stomach are in part mechanical and in part chemical. The mechanical functions are carried out by the contractions of the muscular gastric wall; the chemical functions are carried out through the secretion of a complex fluid, the gastric juice.

The stomachs of both humans and rodents exhibit a single chamber and hence are termed monogastric. However, the stomachs of rodents exhibit two distinctly different, grossly discernible regions: the forestomach (proventriculus) and the glandular stomach. The forestomach differs from the glandular stomach in that the forestomach does not possess a secretory epithelium. Humans do not possess a region comparable to the rodent forestomach.

The luminal surface of the stomach of both rats and humans is characterized by the presence of ridges or rugae (see figure A-4) that serve as a reservoir of epithelium to accommodate the expansion of the stomach during feeding. In addition, the surface is studded with numerous depressions, the gastric pits.

A.3.2 Nutrient Processing

The stomach is the site of the initial phase of digestion of proteins and fats. This is accomplished by enzymes that are secreted by both the salivary glands (lingual lipase) and the stomach itself (pepsinogen which is activated to pepsin). In addition to pepsin, the stomach secretes mucus, hydrochloric acid, intrinsic factor, and electrolytes. This complex mixture is termed "gastric juice."

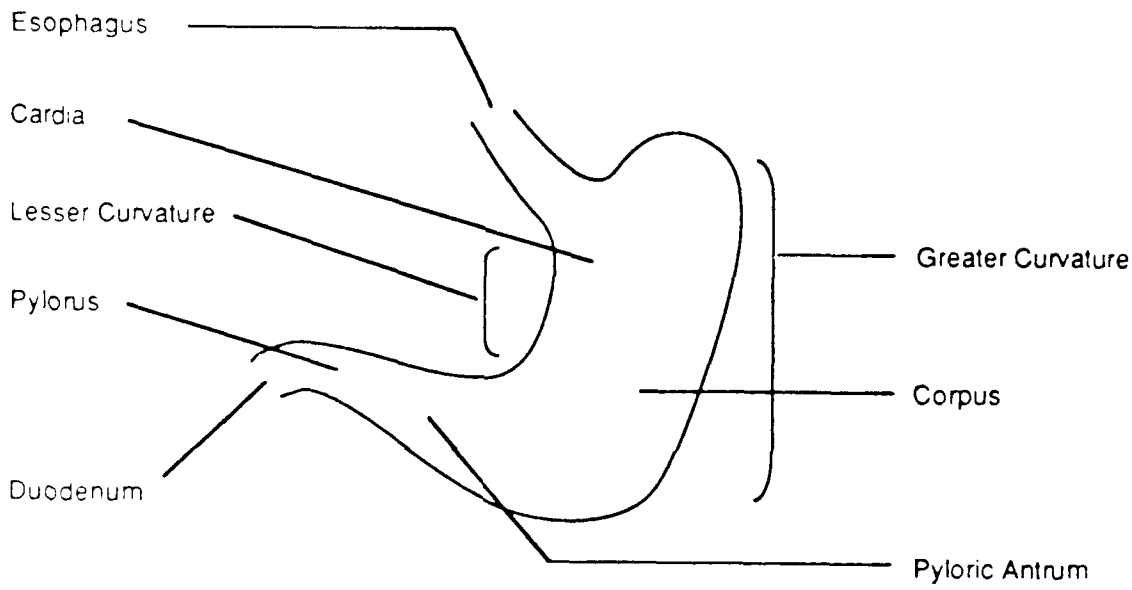


Figure A-2
Gross Anatomical Regions of the Human Stomach

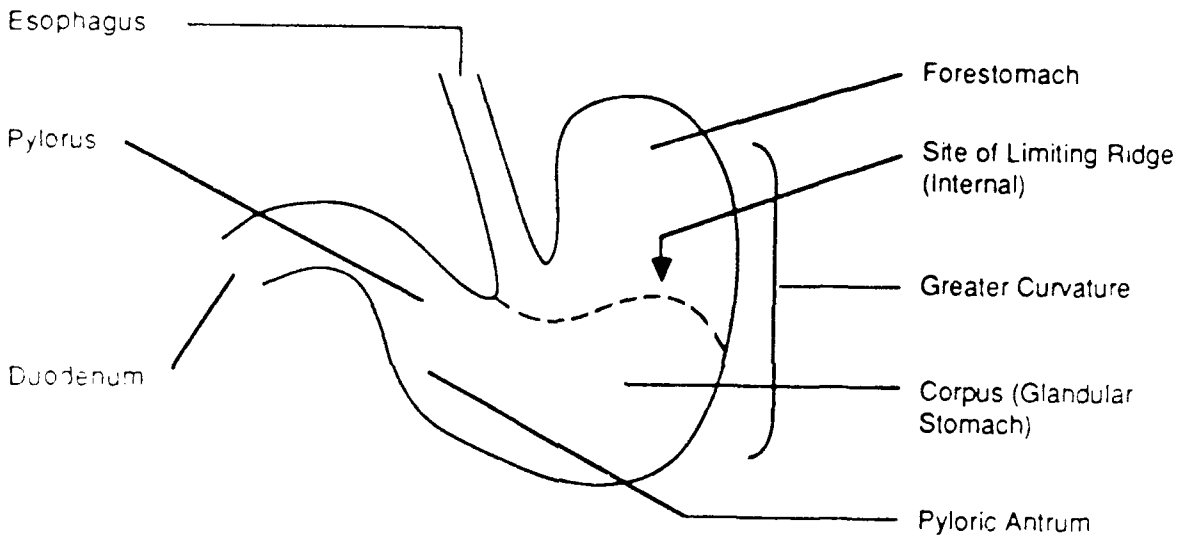
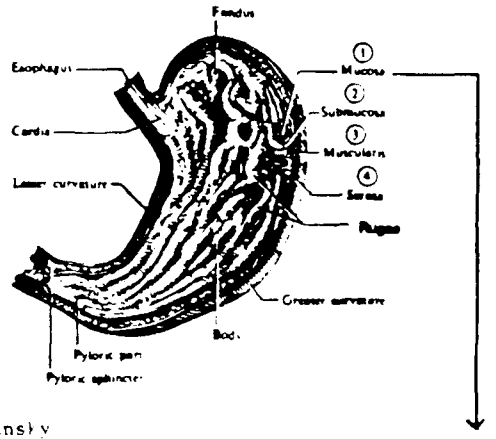
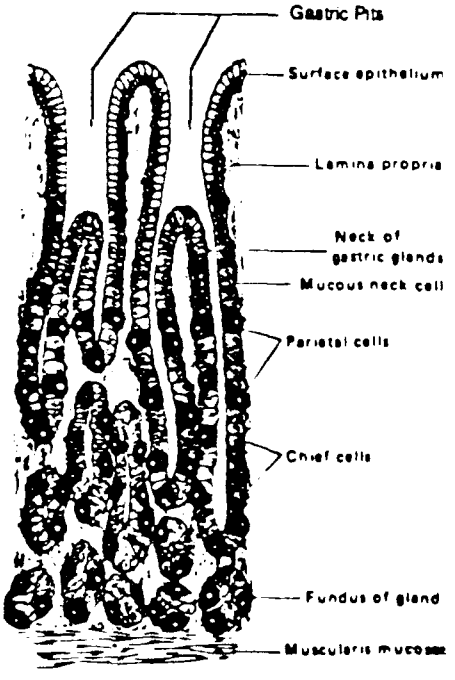


Figure A-3
Gross Anatomical Regions
of the Rat Stomach



Source: Adapted from Pansky and House, 1969



Source: Adapted from Longley et al., 1974

Figure A-4
Internal Structure and Microscopic Anatomy of the Human Stomach

The action of gastric juice on food is enhanced by the muscular contractions of the stomach wall which mix and massage the gastric contents. Solid particles of food are squeezed against the pylorus by the wall of the gastric antrum and then propelled back into the lumen of the stomach. The pylorus closes during the contraction of the antral wall to prevent passage of solid food particles of greater than 0.2 mm diameter from entering the duodenum. In general, liquids are not impeded from leaving the stomach. (See section A.3.8 for more details.) The propulsion of food against the pylorus, followed by the grinding action of the antral wall and subsequent retropulsion of food back into the lumen of the body of the stomach is termed the antral mill. Although the antral mill is active in the digestive process, it does not break up the solid digestible food in a fashion similar to chewing. Rather, the contractions allow digestive juice to come into contact with the digestible matter. As a result of enzymatic and acid hydrolyses, solid nutrient material is converted to a liquid phase termed "chyme." The speed with which liquid chyme passes from the lumen of the stomach depends upon its composition. It should be noted that the energy from the contractions of the stomach is sufficient to emulsify some of the dietary lipid prior to its entry to the small intestine.

A.3.3 Epithelial Characteristics

The luminal surface of the entire stomach of humans and the glandular stomach of rats is lined by a simple columnar epithelium that covers the rugae and extends into the gastric pits (see figure A-4). The apical region of the epithelial cells is filled with mucigen granules that, upon release, give rise to a protective layer of mucus that covers the epithelial surface. Goblet cells (the major source of mucus in the intestines) are notably absent in the stomach. The luminal plasma membrane of the epithelial cells exhibits microvilli; however, the microvilli are not found in sufficient numbers to form a brush border as is characteristic of the absorptive epithelia of the intestines.

The base of each gastric pit serves as the opening for up to seven glands. The glands are simple, branched tubular structures that extend through the underlying lamina propria. In the regions near the entry of the esophagus and the pyloric exit into the small intestine, these glands are predominantly mucous glands. Throughout the rest of the stomach, the gastric glands are responsible

for producing gastric juice. The predominant cell types are the parietal cell (which produces hydrochloric acid) and the chief cell (which produces pepsinogen).

The gastric epithelium is continually desquamated into the lumen. The half-life for surface epithelial cells is estimated to be 12 to 24 hours (Snyder et al., 1975), and the entire mucosal epithelium is thought to be replaced every three days. Mitotic activity is generally restricted to the base of the gastric pits and the necks of the glands.

In addition to the secretory (glandular) portion of the stomach, rats possess a forestomach (proventriculus) that serves as an entry from the esophagus. The epithelial surface of the forestomach is grossly distinct from the corpus of the stomach and is separated from it by a limiting ridge (see figure A-3). The epithelium of the forestomach resembles that of the esophagus; it is a stratified squamous epithelium that is cornified. The forestomach contains no glands and is not considered to be a site of absorption. There is no homologous structure in humans.

A.3.4 Secretory Function

A major activity of the entire gastric epithelium in humans and the glandular epithelium of rats is secretion. The secretory epithelium in different regions is designed to secrete several types of products. As a whole, the stomach elaborates a complex secretion of an alkaline fluid, mucus, pepsinogen, intrinsic factor (vitamin B_{12}-binding factor), and hydrochloric acid. Mucus is elaborated by surface mucous cells on the epithelial surface of the lumen and by the mucous neck cells at the opening of the gastric glands. The mucus acts as a protective coat to the luminal epithelium, inhibiting the corrosive effects of the acid secretion. Pepsinogen is secreted by the chief cells; it is the precursor of the enzyme pepsin that digests protein. The parietal cells, which are located within the gastric glands, secrete both intrinsic factor and hydrochloric acid. The combined pH of the gastric juice in humans is 1 to 2. The pH of the parietal cell secretion at the neck of the gastric glands is approximately 0.7 in humans (Granger et al., 1985).

In humans, both the quantity and the characteristics of the gastric juice can be altered in response to the luminal contents. During the resting (fasted) state, humans usually secrete hydrochloric acid at the rate of 2 to 3 milliequivalents (meq) per hour. Secretion is under both neural

and hormonal control. The neural control is by way of the vagus nerve (cranial nerve X) which is stimulated by distention of the stomach. Hormonal control is through release of the hormone gastrin by cells in the walls of both the stomach and the small intestine that have receptors that are sensitive to the presence of partial digestion products of protein (polypeptides and amino acids). The amino acids tryptophan and phenylalanine are potent stimulators of gastrin release. During a state of maximal secretion, the rate of acid production rises to 10 to 355 mEq per hour (Granger et al., 1985). Under maximal secretion conditions, the stomach can secrete gastric juice at a volume of 700 ml per hour (Guyton, 1971). It has been estimated that stimulation by the vagus nerve is responsible for about 500 ml per hour and gastrin stimulation is responsible for 200 ml per hour. The total daily volume of gastric secretion is approximately 2,000 ml (Guyton, 1971).

The presence of products of lipid digestion in the stomach and small intestine inhibits the release of gastrin and delays gastric emptying. The most powerful inhibitors of gastrin release are monoglycerides and fatty acids; dietary triglycerides are inactive. Only long-chain fatty acids are able to exert the delaying effect on gastric emptying; the optimal chain length is 14 carbons (Granger et al., 1985).

A.3.5 Absorption of Nutrients

Although the primary functions of the stomach are those of secretion of gastric juice and initiation of digestion, the epithelial characteristics of the stomach are compatible with absorption of some nutrients. For instance, the surface cells of the gastric epithelium possess microvilli, which are cell membrane modifications that are commonly found on absorptive cells. Substances that are able to penetrate the mucus layer of the stomach, that possess some degree of lipophilicity, and that exist in nonionized form are able to pass through the cell membranes of gastric epithelial cells and to be absorbed in the stomach. In humans, substances that are absorbed in the stomach include water, ethanol (which has relatively high lipophilicity), and salicylates (which tend to exist in the nonionized form in the acid conditions of the human stomach) (Granger et al., 1985). The extent of absorption in the stomach is likely to be a combination of the amount of time that the substance is in contact with the epithelium and the physical and chemical properties of the substance.

A.3.6 Bacterial Flora

Due to the acid conditions of the human stomach and tendency for the human stomach to empty periodically, bacterial flora are not prevalent in the stomach. Although bacteria are not completely absent from the human stomach, the stomach is considered to be functionally sterile during its normal, healthy state (Borriello, 1984). The number of organisms is estimated to range from $0\text{-}10^3$ organisms per ml of gastric juice (Borriello, 1984) or $0\text{-}10^5$ viable organisms per gram wet weight of tissue (Drasar et al., 1970; Calabrese, 1983). The normal organisms present include fungi, streptococci, and lactobacilli.

The stomach of the rat exhibits a rich population of bacterial flora. This may be due, in part, to the lower acidity of the stomach contents in the rat (forestomach pH 5.0; fundus pH 3.8; Smith, 1965; via Calabrese, 1983). The content of flora for the rat stomach has been estimated to be 10^7 to 10^9 organisms per gram of wet weight of tissue (Drasar et al., 1970; Calabrese, 1983) and has been measured as $10^{8.4}$ organisms per ml of chyme in the forestomach and $10^{7.6}$ organisms per ml of chyme in the fundus (Smith, 1965 via Calabrese, 1983). The organisms present include streptococci, lactobacilli, yeasts, and *Escherichia coli*.

A.3.7 Adventitious Absorption

A variety of nutrients and drugs are known to be absorbed to some degree in the stomach. Fatty acids with medium chain lengths such as octanoic acid and decanoic acid are absorbed from the stomach (Clark et al., 1969). Substances that are weak acids (e.g., many drugs) are not completely ionized at the acid pH conditions found in the stomach and are often well absorbed by the gastric mucosa (Schanker et al., 1957). Some substances such as the anticonvulsant, sodium valproate, are not only well absorbed from the stomach but also may be absorbed at a rate per unit surface area that is higher in the stomach than in the small intestine (Yeomans et al., 1982). Typically, for substances that possess moderate lipophilicity and exist in a nonionized form in the stomach, it is likely that at least some gastric absorption will occur.

A.3.8 Dimensions and Transit Time

In humans, the weight of the empty stomach of an adult male is 150 g (approximately 0.2 percent of the body weight of a 70 kg man). The physiological capacity (volume) of the stomach is 1,300 ml and the surface area of the mucosa is estimated to be 525 cm^2 (Snyder et al., 1975). In rats, the weight of the empty stomach is 0.5 percent of the body weight (Caster et al., 1956). The stomach is divided into two chambers by the limiting ridge, a 2 mm high fold on the luminal surface that divides the stomach into a forestomach (proventriculus) that accounts for 60 percent of the volume of the stomach (Iatropoulos, 1986), and a glandular stomach. It is the presence of the limiting ridge that prevents rats from vomiting (Iatropoulos, 1986).

The contents of the stomach exert some effect on the speed of emptying. For instance, liquids such as water or isotonic saline spend little time in the stomach. In the case of saline, the half-time for residence in the stomach is 12 minutes (Granger et al., 1985). It is thought that emptying of liquids is regulated by the pressure across the pylorus. In dogs (and by extrapolation in other intermittent-feeding mammals), the square root of the volume of liquid remaining after ingestion in an empty stomach, at any time, is a linear function that decreases with time (Kelly, 1974, via Granger et al., 1985).

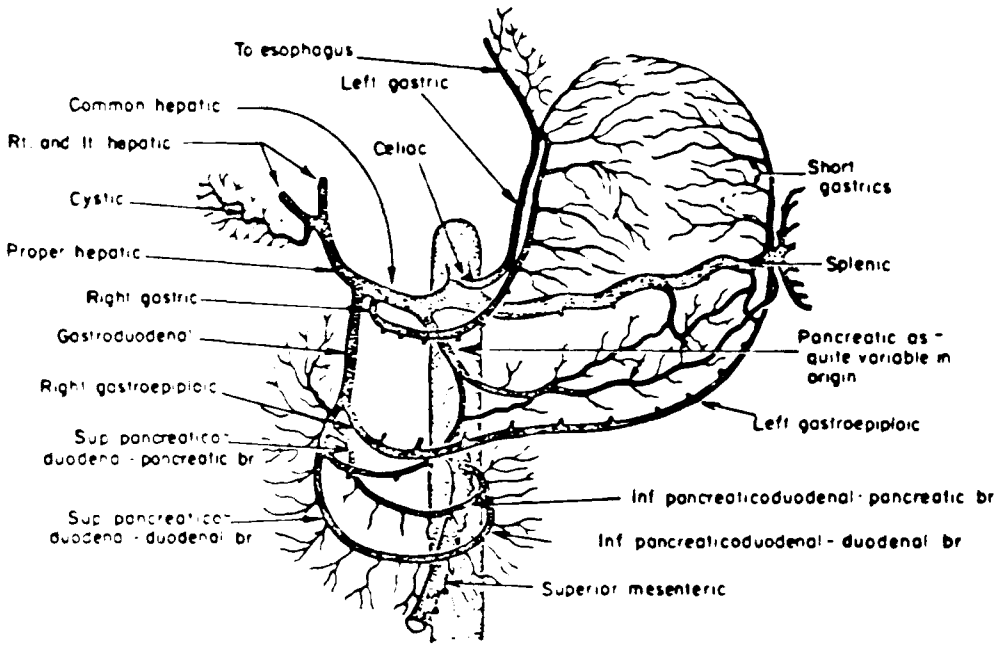
In the case of digestible solids, the pylorus acts as a filter that retains the solids until they are reduced to a particle size of 0.2 mm diameter. The speed with which the chyme that forms around the digestible matter leaves the stomach depends upon the composition of the chyme. Chyme that is high in carbohydrates empties more quickly than chyme that is high in proteins, which in turn, empties more quickly than chyme that is high in lipids. Subsequent to ingestion of a mixed meal, the components of the meal may leave at different times. Hinder and Kelly (1977) reported the times for emptying of various components of mixed meal to dogs. The mixed meal was designed to study the emptying of a liquid, a digestible solid, and an indigestible solid after concurrent ingestion. The meal was comprised of 400 ml of 1 percent dextrose, 50 grams of cubed liver, and 40 plastic spheres. The half-time for emptying of dextrose was about 30 minutes; the half-time of emptying of liver was about 2 hours; none of the plastic spheres had left the stomach at 4 hours after ingestion. Marcus and

Lengemann (1962) reported the half-time for gastric emptying in rats to be one hour after solid food and 36 minutes after liquid ingestion.

A.3.9 Blood Flow

The main blood supply to the stomach is derived from all three branches of the celiac trunk (see figure A-5). The major arteries of the celiac trunk and their branches that supply the stomach are: the right gastric and right gastroepiploic branches of the common hepatic artery; the short gastric and left gastroepiploic branches of the splenic artery; and the left gastric artery. These vessels divide within the walls of the stomach to provide a rich anastomosing network that supplies the mucosa. Anastomoses between the branches of esophageal vessels and the branches of the gastric arteries occur in the wall of the stomach. These anastomoses provide collateral circulation in the case of obstruction of blood flow (the source of esophageal varices seen in hepatic cirrhosis).

It has been estimated that the blood flow through the celiac trunk is 700 ml/min in a 70 kg man with a cardiac output of 6 L/min (Jacobson, 1985; Granger et al., 1985). Blood flow via the "hepatic artery" is estimated to be 500 ml/min. By subtraction, the amount of blood that is supplied to the stomach plus the spleen is estimated to be 200 ml/min under these conditions (Jacobson, 1985). It is not clear, however, just what is meant by the "hepatic artery." As noted above, one major branch of the celiac trunk is the "common hepatic artery" which has three main branches: the right gastric (to the lesser curvature of the stomach and the lesser omentum), the gastroduodenal (a short thick vessel that supplies the proximal duodenum, the superior surface of the pancreas, and the right gastroepiploic branch to the greater curvature of the stomach and the greater omentum), and the "hepatic artery proper." It seems unlikely that 5/7 of the blood flow from the celiac trunk would flow through the hepatic artery proper. The blood flow rate of 500 ml/min may have been measured in the common hepatic artery; if so, the blood flow to the stomach would include the 200 ml/min as figured above (i.e., the blood found in the other two branches of the celiac trunk) plus a portion of the 500 ml/min distributed through the right gastric and gastroduodenal branches of the common hepatic artery.



Source: Crafts, 1979.

Figure A-5
Diagrammatic Presentation
of the Most Common Arrangements
of the Celiac Artery and Its Branches

Venous blood from most regions of the stomach drains into veins that accompany the arteries. These veins drain ultimately into the hepatic portal vein which carries the blood to the liver. In the region of the cardia, the veins of the stomach anastomose with the veins that drain the lower portion of the esophagus. These veins have no valves; consequently, under conditions of increased pressure in the portal system, blood from the stomach can drain by way of the esophageal veins into the tributaries of the venae cavae (azygos veins) which bypass the liver.

The arrangement of arterial supply and major venous drainage routes in rats is the same as described for humans (Hebel and Stromberg, 1986; Chiasson, 1956).

A.3.10 Lymphatic Flow

There is no special arrangement of lymphatic vessels in the wall of the stomach that corresponds to the lacteals of the small intestine. Nevertheless, the stomach has a relatively rich plexus of lymphatic vessels that accompany the corresponding arteries. The lymphatic vessels drain ultimately into the thoracic duct which returns lymph to the venous system at the juncture of the left internal jugular and left subclavian veins in the root of the neck. Substances that are transported by the lymphatic vessels would, therefore, bypass the liver.

A.4 SMALL INTESTINE

A.4.1 General Description and Function

The small intestine is the major site for the digestion and absorption of nutrients, water, and electrolytes into the body. It is a hollow muscular tube that extends from the pyloric sphincter of the stomach to the ileocecal valve which marks the entry into the colon. The small intestine is divided into three unequally sized portions: the duodenum, the jejunum, and the ileum.

The duodenum is the first and shortest portion of the small intestine. It receives the chyme from the stomach as well as the secretions of the major adnexal digestive glands: the pancreas and liver. In addition, the duodenum receives the secretions of the Brunner's glands that are located within its walls.

The jejunum is the second segment of the small intestine. No adnexal digestive glands empty into it. The absorption of most nutrients occurs prior to the time the chyme has traversed this segment of the small intestine.

The ileum is the third and longest segment of the small intestine. Its walls are thinner than those of the jejunum, and aggregations of lymphoid tissue (Peyer's patches) are readily discernible. The exit from the small intestine into the colon occurs at the distal end of the ileum through ileocecal valve.

Throughout its length, the luminal surface of all portions of the human small intestine exhibits crescentic folds of the mucosa (plicae circulares) that project into the lumen. The plicae are oriented perpendicular to the long axis of the small intestine and appear as one-half to two-thirds of a doughnut when observed in cross section. In contrast to the rugae of the stomach, which are reservoirs of mucosa that flatten out when the stomach is distended, the plicae are permanent structures that serve both to increase the surface area for absorption and to retard the passage of chyme. The plicae are largest and most numerous in the duodenum and proximal jejunum; distally they diminish in size and numbers. They are virtually absent in the distal third of the ileum. Rats are among those species that lack plicae circulares (Mayhew, 1984).

A.4.2 Nutrient Processing

The small intestine is the major site of digestion and absorption of nutrients. Digestion is accomplished by enzymes that are secreted by both the intestinal mucosa and the adnexal digestive organs. The principal digestive enzymes, their respective source organs, substrates, and cleavage products are presented in table A-1. Enzymatic activity takes place both in the lumen of the small intestine and at the brush border of the enterocytes as the nutrients traverse the absorptive cell membrane. The results of the enzymatic activity are that dietary carbohydrates are ultimately converted to hexoses; dietary proteins are converted to polypeptides and amino acids; nucleic acids are converted to purine and pyrimidine bases and pentoses; and dietary fats are converted to glycerides, fatty acids, and glycerol.

Table A-1
Principal Digestive Enzymes[a]

Enzyme	Source	Substrate	Products
α-Amylase	Salivary glands	Starch	Smaller carboyhydrate plymers (minor physiologic role)
Lingual lipase	Salivary glands	Fat	Glycerides, fatty acids (minor physiologic role)
Pepsin	Chief cells of stomach	Protein	Polypeptides
Enterokinase	Duodenal mucosa	Trypsinogen	Trypsin
Trypsin	Exocrine pancreas	Denatured proteins and polypeptides	Small polypeptides (also activates chymotrypsinogen to chymotrypsin)
Chymotrypsin	Exocrine pancreas	Proteins and polypeptides	Small polypeptides
Nucleases	Exocrine pancreas	Nucleic acids	Nucleotides
Carboxypeptidases	Exocrine pancreas	Polypeptides	Smaller polypeptides[b]
Pancreatic lipase	Exocrine pancreas	Fat	Glycerides, fatty acids, glycerol
Pancreatic amylase	Exocrine pancreas	Starch	Maltose units
Aminopeptidases	Intestinal mucosa	Polypeptides	Smaller polypeptides[c]
Dipeptidase	Intestinal mucosa	Dipeptide	Amino acids
Maltase Lactase Sucrase	Intestinal mucosa	Maltose Lactose Sucrose	Hexoses (glucose, galactose and fructose)
Nucleotidase	Intestinal mucosa	Nucleotides	Nucleosides, phosphoric acid
Nucleosidase	Intestinal mucosa	Nucleosides	Purine or pyrimidine base, pentose
Intestinal lipase	Intestinal mucosa	Fat	Glycerides, fatty acids and glycerol

[a]Assembled from information in Guyton, 1971; Shepard, 1971; and Ganong, 1979.
[b]Removal of C-terminal amino acid.
[c]Removal of N-terminal amino acid.

The action of the digestive enzymes on chyme is enhanced by the highly orchestrated muscular contractions of the intestinal wall which serve to mix, massage, and slowly transport the chyme in a net aboral direction. In addition to the gross actions of the intestinal wall, the muscularis mucosae (which extends into the plicae circulares) and smooth muscle fibers in the core of the villi can move the plicae and villi to and fro, disrupting the unstirred layer and bringing the chyme into intimate contact with the microvilli of the enterocytes.

A.4.3 Epithelial Characteristics

The luminal surface of the entire small intestine of both humans and rats is lined by a simple columnar epithelium that covers the villi and extends into the crypts of Lieberkuhn. On the villi, the majority of these cells are enterocytes. The luminal surface of the enterocytes is characterized by a dense population of microvilli. It is estimated that each enterocyte has 3,000 to 7,000 microvilli (Granger et al., 1985). The surface of each microvillus is covered by a carbohydrate-rich coat termed the glycocalyx. Interspersed among the enterocytes are a large number of mucus-producing goblet cells; goblet cells are located both on the villi and in the crypts. The crypts are lined with a large number of undifferentiated cells that undergo mitosis and are the precursors of the mature cells. In addition to the enterocytes and goblet cells lining the crypts, there are paracrine and endocrine cells that secrete hormones in response to certain stimuli. For some stimuli such as elevated acidity or osmolarity of luminal contents, or the presence of fats, the secreted hormones slow gastric emptying and gastrointestinal motility (Welling, 1977).

All of the epithelial cells are bound together near their luminal surfaces by tight junctions so that there are no gaps between cells. As is the case with tight junctions throughout the body, the presence of calcium ion is required to maintain the integrity of the junctions (Smith, 1986). Although the cells are adherent to each other, the tight junctions are not completely impervious to the flow of water either into or out of the lumen (Erlij and Martinez-Palomo, 1978; Granger et al., 1985). The epithelium of the small intestine behaves as if there were pores through it; the effective pore sizes decrease from an 8 Å radius in the duodenum and proximal jejunum to 4 Å in the ileum. Some authors imply that the anatomical site of these pores (which is responsible for the rapid transport for fluids) is

the tight junctions while others contend that all transport is transcellular via aqueous channels formed by integral proteins in the enterocyte cell membrane (discussed in Smith, 1986; Granger et al., 1985).

The intestinal epithelium is continually desquamated into the lumen at the tips of the villi. The source of new enterocytes is from the mitotically active precursor cells located in the crypts. The daughter cells of the mitoses move up the crypt walls and up the villi. They differentiate en route into any of the mature epithelial cell types. The time for a daughter cell to journey from the crypt to the villus tip where it is desquamated in about 3 days in the proximal jejunum; the time increases in the terminal ileum due to the greater length of the villi (Granger et al., 1985). Part of the longer duration in the ileum is related to the greater length of the ileal villi compared to those of the proximal jejunum. In humans, the rate of cell loss from the small intestinal epithelium is estimated to be 20 to 50 million cells per minute or about 50 to 250 g of epithelial cells per day (Snyder et al., 1975). The entire epithelial lining of the small intestine is estimated to be replaced every 3 to 4 days (Padykula, 1973).

A.4.4 Secretory Function

Two major activities that take place in the small intestine are the secretion of fluid by the intestinal mucosa and the receipt of fluids secreted by the adnexal digestive organs. This complex fluid that enters the lumen serves several purposes: it helps to dissolve the chyme or to keep it fluid; it is a vehicle for several digestive enzymes; it changes the pH of the chyme or maintains it near the optimum pH for the digestive enzymes; and its mucus component helps to protect the intestinal wall from any excoriative effects of the chyme during its transit. Despite its multiple sources of origin, intestinal juice is isotonic with plasma (Granger et al., 1985).

The fluid that is secreted by the mucosa of the small intestine itself is formed by the epithelial cells at the base of the crypts. This secretion (termed succus entericus) is virtually pure extracellular fluid with a pH of 7.8 to 8.0 (Guyton, 1971). It contains no enzymes except for enterokinase (found only in the duodenum) and a small amount of amylase. In addition to the secretion from the crypts, mucus is secreted into the lumen from the numerous goblet cells located throughout the intestine.

Intestinal secretion is stimulated by the presence of chyme in the lumen; the total daily volume of secretion is about 3,000 ml (Guyton, 1971).

The secretions of the adnexal digestive glands enter the small intestine in the duodenum. The largest volume of secretion is derived from the exocrine pancreas which produces about 1,200 ml of fluid per day (Guyton, 1971). The pancreatic secretion enters the duodenum by a common duct shared with the liver and gall bladder. This duct enters the small intestine at the duodenal papilla, approximately 7 cm from the pylorus. In contrast to the succus entericus, the pancreatic secretion has a rich content of digestive enzymes (see table A-1) and a high content of bicarbonate ion that helps to neutralize the acid chyme as it enters the duodenum from the stomach.

Approximately 700 ml of bile that was formed by the liver and either secreted directly or stored and concentrated in the gall bladder are secreted each day into the duodenum through the common bile duct with the pancreatic secretion (Guyton, 1971). Bile is a complex aqueous solution of electrolytes and organic compounds including cholesterol, bile acids, and phospholipids. The bile acids are amphipathic carboxylic acid derivatives of cholesterol. They combine with phospholipids to form structures known as micelles that are important in the absorption of dietary lipids and substances that might be otherwise insoluble (Weisbrodt, 1985). Concentration of bile in the gall bladder is effected by removal of water and results in acidification of the bile (from pH 8.2 for hepatic bile to pH 6.5) and increases in the concentration of cholesterol, bile acids, cations, and phospholipids without changing its isotonicity (Weisbrodt, 1985; Granger et al., 1985). The reason for the lack of change in tonicity is due to the minimal osmotic activity of micelles, which are formed of bile acids and phospholipids and which sequester the cholesterol and sodium (Granger et al., 1985). Rats, in contrast to humans, do not have gall bladders.

In addition to the normal constituents of bile, the liver can excrete biotransformed xenobiotics into the bile. This enables the liver to detoxify and eliminate xenobiotic substances that were absorbed from the small intestine without returning them to the vascular circulation. In rats, substances that have been biotransformed and possess molecular weights of at least 325 daltons are excreted into the bile (Gregus and Klaassen, 1986; Smith, 1973). However, since bile is eventually secreted into the duodenum, these products of hepatic metabolism may be subject to reabsorption

from the small intestine, giving rise to enterohepatic cycling of the substance. Humans also are capable of enterohepatic cycling of substances of at least 500 molecular weight; however, it is not certain that the molecular weight threshold is as low as in rats because humans, like monkeys, may be poor biliary excreters (Smith, 1973; Hirom et al., 1972; Calabrese, 1983).

The last secretion of adnexal digestive organs to be considered here is that of the Brunner's glands. Brunner's glands are located in the wall of the duodenum, primarily between the pylorus and the entry of the common bile duct. Their secretion is of small volume, approximately 50 ml per day in humans (Guyton, 1971). In humans, Brunner's gland secretion is rich in mucus and high in bicarbonate; it may exhibit a pH as high as 8.9. Despite the small volume, the Brunner's gland secretion is important in protecting the duodenal mucosa from the acid chyme that flows through the pylorus. In contrast to humans, the Brunner's gland secretion of the rat is serous (Iatropoulos, 1986).

In humans, the total daily volume of secretion into the small intestine is 8.0 to 8.5 liters (Guyton, 1971); the daily intake of water is estimated to be 1.5 to 2.0 liters per day. Most of the 10 to 11 liters of fluid that enter the lumen of the small intestine are absorbed; only about 1.5 liters escape into the large intestine via the ileocecal valve (Granger et al., 1985).

A.4.5 Absorption of Nutrients

The small intestine is the major site for the enteric absorption of substances into the body. The anatomical features previously described, including plicae circulares (in humans), villi, and microvilli, serve to increase the area of contact between the luminal contents and the absorptive epithelia. The mechanisms by which nutrients are absorbed include active transport, facilitated transport, passive diffusion, and convection. Xenobiotic materials are likely to be absorbed by the latter two mechanisms only. Most enteric absorption occurs prior to the time the chyme has traversed the proximal half of the jejunum. The lone, major exception to this is for bile salts which are actively transported in the distal portion of the ileum (Granger et al., 1985). The mechanisms of absorption are discussed in detail in appendix B.

A.4.6 Bacterial Flora

Chyme that enters the duodenum from the highly acidic human stomach is essentially sterile. For this reason, the bacterial content of the proximal portions of the healthy human small intestine is low. The number of organisms present has been estimated to range from 0 to 10^5 per gram wet weight of tissue in the proximal small intestine (Drasar et al., 1970; Calabrese, 1983) or 10^2 to 10^4 organisms per ml of chyme of humans (Borriello, 1984). The number of organisms increases as chyme is propelled through the small intestine. In the distal ileum the number of organisms is estimated to be 10^6 to 10^7 organisms per gram wet weight of tissue (Drasar et al., 1970; Calabrese, 1983) or 10^5 to 10^9 organisms per ml of chyme (Boriello, 1984). The normal organisms include lactobacilli, streptococci, enterobacteria, and bacteroides.

In contrast, the small intestine of rats has a rich complement of bacterial flora in the proximal segment. The number of organisms has been estimated to be 10^6 to 10^8 per gram wet weight of tissue (Drasar et al., 1970; Calabrese, 1983) and has been measured as 10^7 organisms per ml of chyme (Smith, 1965, via Calabrese, 1983) in the proximal small intestine. In the distal small intestine, the difference in bacterial flora between humans and rats is not so great. Rats are estimated to have 10^7 to 10^8 organisms per gram wet weight of tissue (Drasar et al., 1970; Calabrese, 1983), and the number of organisms has been measured as 10^8 organisms per ml of chyme (Smith, 1965, via Calabrese, 1983). The organisms present include lactobacilli, streptococci, enterobacteria, and yeasts.

A.4.7 Adventitious Absorption

Most nutrients and many drugs are absorbed enterically. Although many nutrients such as amino acids are actively transported, some nutrients and most drugs are absorbed by diffusion. These substances are typically nonionized at intestinal pH and are both amphipathic and moderately lipophilic. Xenobiotic substances that exhibit similar characteristics will also be absorbed from the small intestine.

A.4.8 Dimensions and Transit Time

In humans, the small intestine of an adult male is estimated to weigh approximately 640 g (empty) and to be approximately 500 cm in length (Snyder et al., 1975). The dimensions of each

subdivision of the small intestine follow. The duodenum is approximately 25 cm in length and weighs 60 g; the jejunum is 190 cm in length and weighs 280 g; and the ileum is 285 cm long and weighs 300 g. The surface area of the small intestine, when calculated as if it were a simple cylinder is approximately 0.33 m^2 (Snyder et al., 1975; Granger et al., 1985). The interior surface of the small intestine is not a smooth walled cylinder, as discussed below.

In humans, three types of anatomical modifications exist that serve to increase the surface area of the small intestine. The first anatomical modification of the luminal surface is the presence of numerous, grossly observable folds of mucosa (plicae circulares, described in section A.4.1) that are oriented orthogonal to the long axis of the small intestine. The plicae circulares are more numerous in the proximal portions of the small intestine as evidenced by the fact that the ratio of luminal surface area to unit length of small intestine is 98 in the proximal jejunum compared to 20 for the terminal ileum. It is estimated that the plicae circulares increase the relative surface area of the small intestine to 1 m^2; an increase of 3 times that of a simple cylinder (Snyder et al., 1975; Granger et al., 1985).

A second modification of the intestinal surface is the presence of numerous microscopic finger-like projections (villi) that extend from the intestinal wall or plicae into the lumen. In humans, the villi of the ileum are longer than those of the duodenum or the jejunum, which helps to counterbalance the decreased frequency of plicae circulares. It is estimated that the villi increase the relative surface area by a factor of 10. (Snyder et al., 1975; Granger et al., 1985).

A third modification of the intestinal surface is observable only with an electron microscope and involves the luminal membrane of the enterocytes. This membrane possesses approximately 3,000 to 7,000 microvilli per cell. These microvilli are responsible for the appearance of the brush border that has been described with the light microscope and increase the surface area by a factor of 20. Together, these modifications of the intestinal mucosa serve to amplify the absorptive surface area to 200 m^2; these surface modifications increase the surface area of the small intestine by a factor of 600X compared to a similarly-sized, simple cylinder (Snyder et al., 1975; Granger et al., 1985).

In rats, Hebel and Stromberg (1986) report the small intestine to be 1,020 to 1,450 mm in length. The length of each subdivision follows. The duodenum is 95 to 100 mm in length; the

jejunum is 900 to 1,350 mm long; and the ileum is 25 to 35 mm long. The luminal surface area of the rat small intestine has been calculated by Holt et al. (1984) to be approximately 100 cm^2 if it is assumed to be a simple cylinder. The surface area is increased to 500 cm^2 by the presence of villi (a factor of 5X); and the surface area is estimated to be approximately 1.0 m^2 (a factor of 20) when the presence of the microvilli are taken into consideration. It should be noted that the amplification of surface area in rats is not so large as that in humans because rats do not possess plicae circulares. Furthermore, despite the fact that intestinal villi of rats are about twice as long as those of man or nonrodent species (Iatropoulos, 1986), the increase due to villus amplification is smaller than the 10-fold amplification in humans. A recent morphometric analysis of the rat small intestine has confirmed the smaller magnitude of the villus amplification. Mayhew (1984) determined the villus amplification to be approximately 5.8 to 6.3X the surface of a simple cylinder, which is in good agreement with the assumptions made by Holt et al. (1984).

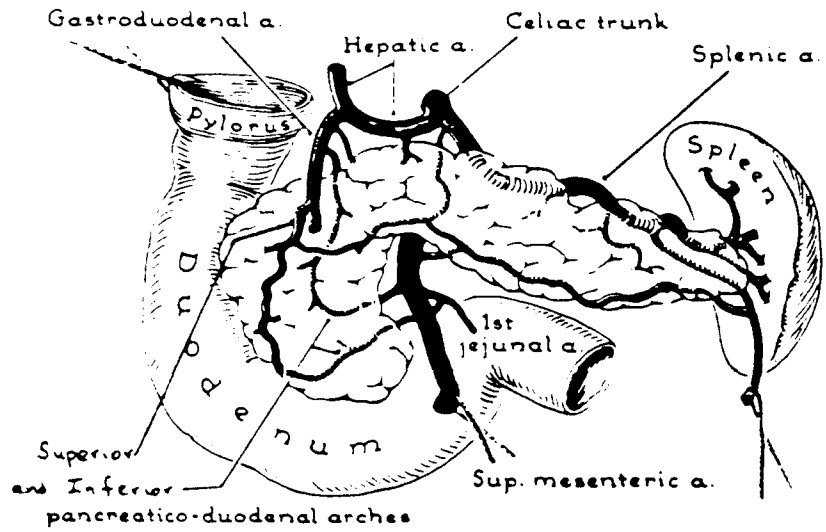
As in the case of the stomach, the contents of the small intestine exert some effect on the speed with which the luminal contents traverse that organ. The muscular walls of the small intestine are subject to segmental and peristaltic contractions that serve to move the contents to and fro, thereby kneading them. In addition, the muscularis mucosae contracts, thereby mixing the unstirred layer and enhancing the contact of chyme with the epithelium. Although the peristaltic contractions may move chyme in either direction within the small intestine, the net movement of chyme in humans is in the aboral direction at a rate of approximately 4 cm/min in the duodenum decreasing to approximately 1 cm/min in the ileum (Granger et al., 1985). Under normal conditions, chyme traverses the human small intestine in 3 to 4 hours. In rats, Marcus and Lengemann (1962) calculated the movement of chyme to decrease from 4 cm/min in the duodenum to about 0.25 cm/min in the ileum. The mean transit time was 3.3 hours. Although these authors reported transit times for each subdivision of the rat small intestine, the subdivisions chosen were equal lengths of the small intestine rather than accurately identified, anatomically distinct regions.

A.4.9 Blood Flow

The major source of blood supply to the small intestine is derived from branches of the superior mesenteric artery. The lone exception to this is the duodenum which receives blood supply

by way of both the superior pancreaticoduodenal artery (derived from the gastroduodenal branch of the celiac axis) and the inferior pancreaticoduodenal artery (a branch of the superior mesenteric artery) (see figure A-6). These vessels reach the small intestine by traveling within the intestinal mesentery within which they divide into branches which run parallel to each other and send smaller branches laterally to form vascular arches with other lateral branches. When they reach the wall of the small intestine, the vessels divide within the walls to provide a rich anastomosing network of arterioles and capillaries that supplies the mucosa. The arterioles traverse the lamina propria and extend throughout the mucosa, including vascularization of the plicae circulares. The arterioles give off smaller branches and capillaries that extend into the villi where they form a capillary bed subjacent to the basement membrane of the enterocyte epithelium.

The rate of blood flow through the superior mesenteric artery in a 70 kg man with a cardiac output of 6 L/min has been estimated to be 700 ml/min (Jacobson, 1985; Granger et al., 1985). This is not the blood flow to the small intestine, however, since the superior mesenteric artery has major branches that supply the proximal portions of the large intestine (cecum, ascending and transverse colons). In addition, there is some blood flow derived from the celiac axis via the superior pancreaticoduodenal artery. The resting rate of blood flow in most gastrointestinal tissues is estimated by many authors to be 50 ml/min/100 g of tissue (Granger et al., 1985); however, the presence of chyme in the lumen can increase the rate of blood flow by 130 percent over the resting rate (Granger et al., 1985). It is not clear whether the term gastrointestinal tissues refers to the mucosa of the small intestine or to the entire intestinal wall. If it refers to the mucosa, the estimate appears reasonable; however, if the estimate refers to the entire wall, the estimate is inaccurate. The reason for the inaccuracy is based upon the anatomical features of the luminal mucosa. The human duodenum and proximal jejunum possess numerous plicae circulares that are comprised of folds of mucosa that increase the amount of mucosa present per unit length of intestine. This means that for a given weight of duodenal wall there would be more mucosa (with its large blood supply) than in the same weight of ileal wall where plicae circulares are essentially absent.



Source: Adapted from Anderson, 1978.

Figure A-6
Diagram of the
Blood Supply to the Duodenum

The capillary bed of the small intestine has some anatomical features that are conducive to absorption. The capillaries that traverse the lamina propria are located only a few microns beneath the basement membrane of the enterocyte epithelium. Each capillary is a tube made of a single, attenuated endothelial cell overlain by a basement membrane of its own. The center of the tube is the conduit for the blood. The distance between the capillary bed and the intestinal lumen is short and the barrier between the chyme and the blood stream is comprised of the enterocyte, the underlying basement membrane, a small (few microns at most) layer of lamina propria, the basement membrane of the endothelial cell, and the thin wall of the endothelial cell itself. In addition to the above features, the endothelial cells of the small intestine capillaries possess numerous openings (fenestrations) that face the lumen and are overlain only by the endothelial basement; at the site of the fenestrations, there is no endothelial cytoplasm to be traversed. The size of the fenestrations is estimated to be 500 Å.

Venous blood from the small intestine drains into veins that accompany the arteries. These veins drain ultimately into the hepatic portal vein which carries the venous blood (containing absorbed substances) to the liver. The flow of blood from the intestine to the liver is known as enterohepatic circulation and is the first leg of enterohepatic cycling. Enterohepatic cycling occurs when materials that have been absorbed by the small intestine and transported to the liver are metabolized by the liver and excreted into the bile. The bile subsequently traverses the biliary ducts and enters the duodenum as described previously. The metabolized substance may be reabsorbed by the small intestine; if so, the enterohepatic cycle just described will be repeated. Enterohepatic cycling of hepatic toxicants or carcinogens can increase the apparent target tissue dose of the toxicant due to multiple cycles through the liver.

The arrangement of arterial supply and major venous drainage routes in rats is the same as described for humans (Hebel and Stromberg, 1986; Chiasson, 1958).

A.4.10 Lymphatic Flow

The arrangement of lymphatic vessels in the small intestine is unique. At the center of each villus, extending parallel to its long axis, is a blind-ending, sac-like lymphatic vessel termed a lacteal.

The lacteals from each villus drain into lymphatic vessels deeper in the wall of the small intestine. The lymphatic vessels accompany the corresponding arteries within the mesentery, but eventually converge upon a large, dilated lymphatic vessel (the cisterna chyli) that is located on the posterior wall of the abdomen between the aorta and the vertebral column. Lymphatic vessels from the entire gastrointestinal tract converge at the cisterna chyli, which is the origin of the thoracic duct. The thoracic duct returns lymph to the venous at the juncture of the left internal jugular and left subclavian veins in the root of the neck. Absorbed substances that are transported by the lymphatics will, therefore, bypass the liver.

The structure of the lymphatic capillaries (lacteals) differ from the structure of blood vascular capillaries in several important ways. While both types of capillaries are comprised of a single layer of endothelial cells, the lymphatic capillaries are located deeper in the lamina propria the blood capillaries, such as chylomicrons with diameters as large as 6,500Å, to be readily absorbed by lacteals (Granger et al., 1985).

The resting rate of lymphatic flow from the small intestine in humans is estimated to be about 0.095 ml/min/100 g of tissue, which is only 0.1 percent of the resting blood flow through the same tissue (Jacobson, 1985). While the 1,000-fold difference in perfusion rates may seem sufficient to discount the importance of lymphatic flow in the transport of absorbed material, it must be borne in mind that (1) the total daily lymphatic flow amounts to 1 to 2 liters per day (Granger et al., 1985), (2) some materials such as dietary lipid (in the form of chylomicrons) are transported only by the lymphatic vessels, and (3) at times of maximal intestinal absorption, as much as 20 percent of the absorbed material is transported by the lymphatic system (Jacobson, 1985).

A.5 LARGE INTESTINE

A.5.1 General Description and Function

The large intestine is a major site for the absorption of water and electrolytes into the body. It is a hollow muscular tube with a wide diameter in its proximal regions; it derived its name from this broad diameter relative to that of the small intestine. The large intestine extends from the ileocecal valve to the anus. The large intestine is divided into several regions. In humans these include the

cecum, ascending, transverse, descending and sigmoid colons, and the rectum (depicted in figure A-7).

The human large intestine exhibits several gross features besides its large diameter that distinguish it from the small intestine. Externally, these include the segregation of the outer longitudinal layer of smooth muscle into three discrete bands called the teniae coli (see figure A-7); sacculations of the intestinal wall called haustra that are caused by the fact that the teniae are shorter than the gut; and small fat-filled sacs of peritoneum attached along the teniae (appendices epiploicae). Internally, the luminal surface possesses folds of mucosa that occur between adjacent haustra (plicae semilunares). The plicae semilunares are neither so numerous nor so large as the plicae circulares of the small intestine.

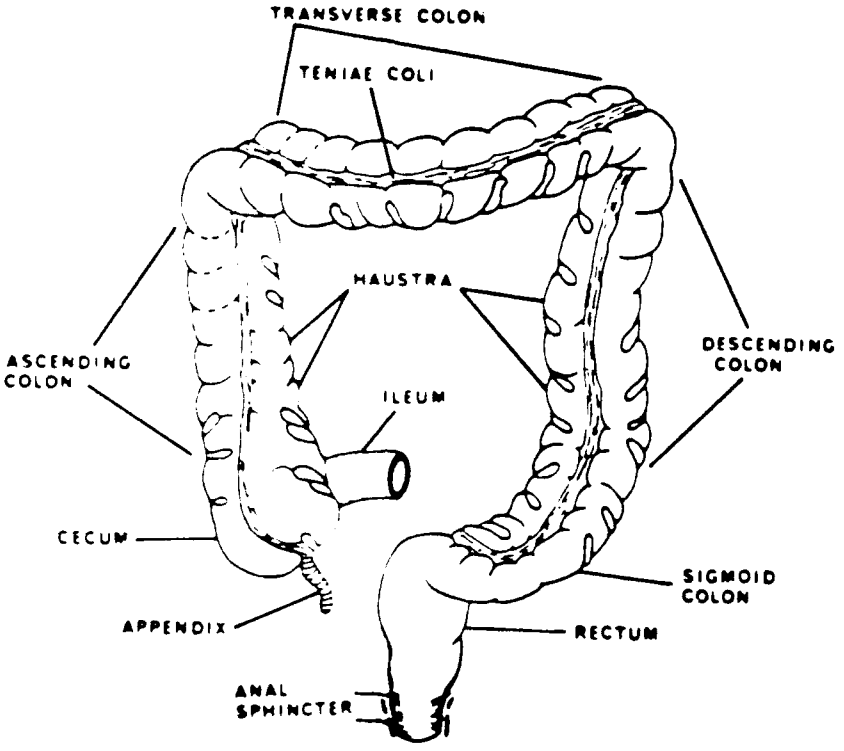
The appearance of the rat large intestine differs markedly from that of the human large intestine. In rats, the outer longitudinal smooth muscle remains a continuous sheet. Thus, rats do not possess teniae coli, haustra, nor appendices epiploicae. The external surface of the rat large intestine is relatively smooth walled. In addition, since rats are quadrupeds and do not have a well developed iliac fossa (the false pelvis), the descending colon enters the true pelvis directly. This means that rats do not have a segment of colon that is designated the sigmoid colon; this is compensated for by a relatively longer rectum. The rat rectum is analogous to the combined human sigmoid colon and rectum.

A.5.2 Nutrient Processing

Little nutrient processing occurs in the large intestine as the result of direct activity of the intestinal mucosa or its modest volume of daily secretion (~60 ml). The nutrient processing that does occur is the result of bacterial metabolism of the contents (see section A.5.6).

A.5.3 Epithelial Characteristics

The luminal surface of the entire large intestine of both humans and rats is lined by a simple columnar epithelium that extends into the intestinal crypts. On the luminal surface the majority of these cells are enterocytes, but they do not possess as many microvilli as are present on the enterocytes of the small intestine (Granger et al., 1985). Interspersed among the enterocytes are a



Source: Adapted from Granger et al., 1985.

Figure A-7
The Human Large Intestine

large number of mucus-producing goblet cells; goblet cells are located both on the surface and in the crypts. Goblet cells are far greater in number and make up a much larger proportion of the luminal epithelium in the large intestine than in the small intestine. The crypts contain a large number of undifferentiated cells that undergo mitosis and are the precursors of the mature cells. In addition to the enterocytes and goblet cells lining the crypts, there are paracrine and endocrine cells that secrete hormones in response to certain stimuli (Welling, 1977).

All of the epithelial cells are bound together near their luminal surfaces by tight junctions so that there are no gaps between cells. As is the case with tight junctions throughout the body, the presence of calcium ion is required to maintain the integrity of the junctions (Smith, 1986). The cells of the large intestine appear to be more tightly bound together since the large intestinal epithelium is more impervious to the convection of water than is the small intestinal epithelium. Still, the tight junctions are not completely impervious to the flow of water either into or out of the lumen (Erlij and Martinez-Palomo, 1978; Granger et al., 1985). As in the case of the small intestine, the epithelium of the large intestine behaves as if there were pores through the enterocyte membrane; however, the effective pore size is decreased to only a 2.3Å radius compared to an 8Å radius in the duodenum and jejunum or 4Å in the ileum (Smith, 1986; Granger et al., 1985).

The large intestinal epithelium is continually desquamated into the lumen. The source of new enterocytes is from the mitotically active precursor cells located in the crypts. The daughter cells from the mitoses move up the crypt walls to the surface. They differentiate en route into any of the mature epithelial cell types. The time for a daughter cell to journey from the crypt to the surface where it is desquamated is about 3 to 6 days (Granger et al., 1985). The entire epithelial lining of the large intestine is estimated to be replaced every 6 to 8 days (Granger et al., 1985).

A.5.4 Secretory Function

Secretion is not a major activity of the large intestine. The total daily volume of large intestinal secretion in humans is estimated to be only about 60 ml (Guyton, 1971). Much of this secretion is the product of goblet cell secretion of mucus. The enterocytes also secrete a small volume of watery secretion that is similar to the small intestinal succus entericus. The large intestinal

secretion is slightly alkaline (pH 8.0) and is virtually devoid of enzymes (Guyton, 1971). Its primary function is the protection of the epithelium. The mucus secretion overlies the epithelium where it protects against (1) the excoriative effects of the fecal matter as it is propelled through the colon and (2) the corrosive effects that could be caused by the acid products of bacterial metabolism.

A.5.5 Absorption of Nutrients

Although the large intestine is not considered to be a major site of digestion, it is an important site of absorption for water and electrolytes. Despite its smaller surface area than the small intestine, the large intestine is more efficient in the absorption of water, sodium, and potassium (Granger et al., 1985). These substances are actively transported out of the lumen in the proximal regions of the large intestine. The epithelium of the distal large intestine (especially the rectum) is relatively impermeable to the flow of water and electrolytes. Consequently, of the 1.5 L of water that enters the large intestine, only 100 to 150 ml is found in the daily fecal mass, and the fluid is hypotonic with respect to plasma (Granger et al., 1985).

Substances in the lumen of the large intestine that have physical and chemical properties that are compatible with absorption will be absorbed. These substances include those substances that have escaped from the ileum to the large intestine without being absorbed or substances that are formed as the result of metabolic activity of the bacterial flora. The latter substances may be the result of further digestion of carbohydrates and lipids or degradative reactions of bile acid metabolites resulting in the re-uptake of previously excreted materials (Drasar et al., 1970; Williams, 1972; Scheline, 1973; Granger et al., 1985).

A.5.6 Bacterial Flora

The large intestine of both humans and rats has abundant bacterial flora. In humans, the number of organisms has been estimated to be 10^7 to 10^{10} per gram wet weight of tissue and 10^{10} to 10^{11} organisms per gram of feces (Drasar et al., 1970; Calabrese, 1983). In rats, the number of organisms was estimated to be 10^8 to 10^9 per gram wet weight of large intestine and 10^9 to 10^{10} per gram of feces (Drasar et al., 1970; Calabrese, 1983). In both humans and rats there are hundreds of species, including both aerobes and anaerobes. In human colons, more than 400 bacterial species are

normally present; anaerobes exceed aerobes by a factor of 10^2 to 10^4 (Granger et al., 1985). The most common aerobes are *Escherichia coli*, lactobacilli, and enterococci; the most prevalent anaerobes are bacteroides and eubacteria.

The metabolism of the colonic bacteria can have an effect on the absorption of materials due to digestive and detoxification reactions. The detoxification reactions may lead to re-absorption of previously conjugated and excreted materials as part of the enterohepatic cycling mechanism.

A.5.7 Adventitious Absorption

Most nutrients and many drugs are absorbed enterically. Although many nutrients such as amino acids are actively transported, some nutrients and most drugs are absorbed by diffusion. These substances are typically nonionized at intestinal pH and are both amphipathic and moderately lipophilic. Xenobiotic substances that escape from the ileum and exhibit similar characteristics will also be absorbed from the large intestine.

In addition, the luxuriant bacterial flora of the large intestine are capable of metabolizing both foodstuffs that escape from the small intestine (Granger et al., 1985) and xenobiotics (cf reviews by Drasar et al., 1970; Scheline, 1973) to products that are more readily absorbed than the parent substance.

A.5.8 Dimensions and Transit Time

In humans, the large intestine of an adult male is estimated to weigh approximately 370 g (empty) and to be approximately 160 cm in length (Snyder et al., 1975). The dimensions of each subdivision of the large intestine follow. The upper large intestine (which receives blood supply from branches of the superior mesenteric artery) weighs 210 g and is 75 cm in length. The upper large intestine is comprised of the cecum (7 cm in length), the ascending colon (18 cm in length), and the transverse colon (50 cm long; 120 g). The combined weight of the cecum and ascending colon is 90 g. The lower large intestine (which receives blood supply from the inferior mesenteric artery) weighs 160 g and is 85 cm long. The lower large intestine is comprised of the descending colon (40 cm long; 90 g), the sigmoid colon (40 cm long), and the rectum (15 cm long). The combined weight of the sigmoid colon and rectum is 70 g. The diameter of the large intestine is not constant

throughout its length. It is widest at its proximal portion and narrows distally. Thus, the diameter of the cecum is about 8.5 cm, whereas the diameter of the sigmoid colon is only 2.0 to 2.5 cm.

The interior surface of the large intestine is smoother than that of the small intestine. The enterocytes do not possess as many microvilli as the enterocytes of the small intestine. There are grossly observable folds of mucosa between haustra (plicae semilunares, described in section A.5.1) and deep crypts in the mucosa. Together, these anatomical features serve to increase the surface area of the large intestine by an estimated 10 to 15 percent over that of a similarly sized cylinder (Granger et al., 1985).

In rats, Hebel and Stromberg (1986) reported the large intestine to be 220 to 260 mm in length. The lengths of each subdivision follow. The cecum is 50 to 70 mm in length; the colon (combined ascending, transverse, and descending colons) is 90 to 110 mm long; and the rectum is 80 mm long. Due to the shallowness of its iliac fossae (the cavity formed by the pelvis), the rat does not possess a sigmoid colon. The length of the rectum probably incorporates the extent of large intestine that is analogous to the human sigmoid colon and rectum. Rats do not possess plicae semilunares.

The transit time of chyme through the large intestine is considerably slower than through other portions of the alimentary canal. In humans on a normal western diet, transit time is 3 to 4 days. As in the case with other regions of the digestive tract, the luminal contents can affect transit time. Individuals consuming large amounts of bran (fiber) in their diets can reduce the transit time through the large intestine to 2 days (Granger et al., 1985).

A.5.9 Blood Flow

The primary sources of blood supply to the large intestine are branches of the superior mesenteric artery (to the cecum, ascending and transverse colons) and the inferior mesenteric artery (to the descending and sigmoid colons and the upper one-third of the rectum). As in the case of the small intestine, these vessels reach the large intestine by traveling within mesenteries of the appropriate segment of the colon. While some vascular arches are formed, they are not so prominent as in the small intestine and they form close to the wall of the large intestine. Since the large intestine does not possess mucosal villi, the distribution of arterioles and capillaries is more similar to that of

the stomach than that of the small intestine. Arterioles traverse the lamina propria to supply the mucosa and extend into the regions of the plicae semilunares. Capillary beds are located a few microns beneath the basement of the enterocyte epithelium, but the endothelial cells do not possess fenestrations.

Venous blood from the large intestine drains into veins that accompany the arteries. These veins drain ultimately into tributaries of the hepatic portal vein which carries the blood to the liver.

The rate of blood flow through the inferior mesenteric artery in a 70 kg man with a cardiac output of 6 L/min is estimated to be 400 ml/min (Jacobson, 1985; Granger et al., 1985). This is not the total blood flow to the large intestine because the upper large intestine receives its blood supply from branches of the superior mesenteric artery.

Another source of blood supply to the distal large intestine is by way of the inferior and middle rectal arteries that are ultimately derived from the internal iliac artery. As in the case of the superior and inferior mesenteric arteries, veins accompany the corresponding arteries. This provides the basis for another anastomosis between the portal and systemic venous systems that may hypertrophy during cases of portal obstruction. The hypertrophied veins are called hemorrhoids and are similar to the esophageal varices described in section A.2.9.

A.5.10 Lymphatic Flow

There is no special arrangement of lymphatic vessels in the wall of the large intestine that corresponds to the lacteals of the small intestine. Nevertheless, the large intestine has a relatively rich plexus of lymphatic vessels that accompany the corresponding arteries. The lymphatic vessels drain ultimately into the thoracic duct which returns lymph to the venous system at the juncture of the left internal jugular and left subclavian veins in the root of the neck. Substances that are transported by the lymphatic vessels would, therefore, bypass the liver.

APPENDIX B

MECHANISMS AND SITES OF NUTRIENT ABSORPTION

This appendix will describe physiological mechanisms by which the various classes of nutrients are absorbed. The general mechanisms will be defined, followed by a more specific description of the mechanisms by which the individual nutrient classes are absorbed.

B.1 MODES OF TRANSPORT

Throughout biology, the cell surface membrane is designed as a water-insoluble barrier to the indiscriminate passage of water-soluble components. The barrier properties of the cell-surface membrane allow the aqueous processes of life to be contained within the cell and the cell contents to be regulated by the various pumps and carriers in the membrane. The cell membrane is water-insoluble by virtue of its being constructed of lipids and lipid-soluble proteins, and it is therefore relatively permeable to lipid-soluble compounds. The gastrointestinal epithelium is a barrier whose properties generally reflect those of the cell-surface membrane. Several modes of transport exist for the uptake of nutrients from the GI tract. Passive diffusion, facilitated diffusion, active transport, and pinocytosis are cellular mechanisms which occur in virtually all living cells and tissues. Convection and enterohepatic cycling are more specific mechanisms which arise from the anatomy and physiology of the GI tract.

B.1.1 Passive Diffusion

Passive diffusion is the simplest of transport mechanisms, the result of the tendency of the random movement of molecules to equalize a concentration gradient between adjacent zones or compartments. Its rate is governed by the steepness of the concentration gradient and the permeability of the barrier being traversed. Lipid-soluble components can diffuse more rapidly through the lipid portion of cell membranes than water-soluble compounds. Passive diffusion is not saturable, which is to say that its rate increases without limit with increasing steepness of the concentration gradient. The rate of passive diffusion exhibits a first-order dependence on the

concentration gradient, and net movement of material stops when the concentrations on either side of the membrane are equal.

B.1.2 Facilitated Diffusion

The transport across membranes of a large number of different compounds of physiological significance is facilitated by the presence in membranes of carrier proteins that specifically bind the compound and move it through the membrane. This process is known as facilitated diffusion. It differs from passive diffusion through a membrane in that the carrier increases the permeability of the membrane for the compound that it binds and also in the fact that the system is saturable. Saturation occurs when at some limiting concentration of compound all of the carrier molecules are continuously occupied and no further increase in the rate of transport will occur as the concentration increases above this point. As with passive diffusion, net transport of a compound by facilitated diffusion will cease when the concentrations on either side of the membrane are equal.

B.1.3 Active Transport

The distinctive feature of active transport is the expenditure of energy to transport a specific compound across a membrane against a concentration gradient. That is, the compound is transported from a compartment of lesser concentration to a compartment of greater concentration. This process goes against the driving force of chemical potential, and is therefore energetically unfavorable, which is to say that energy must be expended in its accomplishment. Active transport is accomplished by a specific carrier protein in the membrane whose binding site defines the compound to be transported and which is capable of coupling expenditure of energy to the transport process. The source of the required energy may be the hydrolysis of ATP, or the movement of another compound down a concentration gradient, that is, from a greater concentration to a lesser concentration. Active transport, like facilitated diffusion, is saturable as the binding capacity of the total number of carrier molecules is exceeded.

B.1.4 Pinocytosis

Pinocytosis is the inward folding of cell surface membrane to form a closed vesicle on the inside of the cell, engulfing a portion of the fluid that bathes the outer cell surface. This process

results in non-selective internalization of materials contained in fluid external to the cell. It is the only means by which large molecules can enter the cell. Pinocytotic vesicles are visible in histologic preparations of intestinal epithelium, but the significance of this process in the absorption of nutrients is not known.

B.1.5 Convection

Convection is the term applied to movement of solutes that follow the flow of water as it flows across the luminal membrane in response to osmotic gradients established by the active transport of nutrients. Compounds transported by this mechanism must be small enough to fit through the pores that are traversed by water.

B.1.6 Enterohepatic Cycling

Bile salts and other components of bile are secreted by hepatocytes into the bile ducts of the liver, collected in the gall bladder, and secreted from the gall bladder into the duodenal lumen in response to ingested fat. Most of the bile salts (80 to 90 percent) are reabsorbed in the distal ileum by a sodium-driven active transport mechanism. They then enter the portal circulation and are returned to the liver for incorporation into bile and resecretion. The total body pool of bile salts is cycled in this way from 4 to 12 times per day. This process is known as enterohepatic cycling. Other components that are absorbed from the gut and that enter the liver through the portal circulation face the possibility of enterohepatic cycling if they are taken up by hepatocytes and secreted into the bile ducts.

B.2 TRANSPORT OF NUTRIENTS

This section will discuss specific mechanisms that have been demonstrated to be operative in the absorption of various nutrient classes. The normal uptake of dietary nutrients occurs predominantly in the small intestine. The absorption of nutrients from the intestinal lumen to plasma or lymph involves the crossing of three membranous barriers. The first barrier is the immediate lining of the intestinal lumen, the brush border membrane of the enterocyte. Entry into the enterocyte through the brush border membrane is the first step in absorption from lumen to capillary. The brush border membrane is relatively impermeable to water-soluble compounds and contains several specific

carrier molecules which bind and transport nutrients. The "backside" of the enterocyte surface that does not face the intestinal lumen is known as the basolateral membrane. This is the second barrier to be crossed as nutrients move from the cytoplasm of the enterocyte to the interstitial space of the submucosa. The basolateral membrane is thinner and more permeable than the brush border membrane. An alternative to passage through the enterocyte is passive diffusion from the lumen through the spaces between the cells (intercellular junctions) directly into the intercellular space which is continuous with the interstitial space. The final barrier between the interstitial space and plasma or lymph is the capillary wall, which contains large pores or fenestrations which allow relatively unhindered passage.

B.2.1 Water and Electrolytes

Absorption of water from the intestine occurs primarily by passive movement in response to osmotic gradients. The uptake of electrolytes and other nutrients increases the osmolarity of the submucosa relative to the lumen, and water flows from the lumen to correct this imbalance. Flow is through pores in the enterocyte membrane and through the intercellular junctions. Pores of 8 Å in radius are located in the duodenum and jejunum, presenting less resistance to water flow than the pores of the ileum, which have radii of 4 Å. A total of approximately 8 to 10 liters of water enter the GI tract in a day. Food or drink include 1 to 2 liters and the remainder is from digestive secretions. Most of the water that enters the GI tract is absorbed. Approximately 0.5 to 1.0 liters are excreted.

Flow of water to correct an initial osmotic imbalance created by either a hypotonic or a hypertonic meal is relatively rapid (half-time of approximately 3 min). Isotonicity is achieved relatively rapidly and maintained throughout the absorptive process by the passive uptake of water which accompanies the uptake of nutrients.

The uptake of sodium ion from the lumen to plasma is driven by a Na^+,K^+-ATPase which is located exclusively on the basolateral side of the enterocyte and pumps sodium out of the enterocyte into the interstitium against a steep concentration gradient. The sodium ion concentration inside of the enterocyte is maintained at 15 mM compared to the concentration of 140 mM external to the cell, both in the interstitial space and in the lumen. The luminal concentration of sodium ion is maintained

approximately equal to the interstitial concentration by passive flow of water through cellular junctions. The movement of sodium ion down the concentration gradient from lumen to enterocyte occurs by passive diffusion through aqueous pores and also by means of carrier molecules in the brush border membrane of the enterocyte which couple the favorable movement of sodium down its concentration gradient to the active transport of sugars, amino acids, and other compounds against a concentration gradient. The gradient of sodium concentration between lumen and enterocyte, maintained by the basolateral Na^+,K^+-ATPase, is thus a critical source of energy for the active uptake of nutrients from the lumen.

The predominant mode of sodium ion uptake differs between the jejunum and the ileum. The jejunum contains aqueous pores of 8 Å in radius, which allow passive diffusion of sodium ions (diameter 5.12 Å) to predominate in this area of the intestine. In the ileum, pore size is smaller, with a radius of 4 Å, which restricts the passive diffusion of sodium and membranous carrier molecules become the predominant mode of sodium ion uptake. The predominance of this mode of transport in the ileum corresponds with the need for sodium-coupled active transport of nutrients in this portion of the intestine, as the last traces of nutrients are removed from the lumen against a concentration gradient.

An average of approximately 600 mEq of sodium per day is absorbed from the GI tract. Diet and digestive secretions each provide approximately one-half of this amount. Sodium absorption is very efficient and less than 1 percent is excreted.

Potassium ion is absorbed primarily by passive diffusion through the cellular junctions of the jejunum in response to a concentration gradient. The Na^+,K^+-ATPase in the basolateral membrane of the enterocyte pumps potassium ion out of the intercellular space and into the cell against a concentration gradient. The decreased concentration of potassium ion that results in the intercellular space favors the passive influx of potassium ion through intercellular junctions. Total daily intake of potassium is approximately 50 mEq per day. The concentration of potassium ion increases as materials move through the intestinal tract.

The uptake of other electrolytes from the lumen occurs by a variety of processes. Chloride ion is absorbed by both active and passive mechanisms in response to concentration and electrochemical gradients. Bicarbonate is absorbed as CO_2 after neutralization. The divalent calcium ion is absorbed into the enterocyte by facilitated diffusion across the brush border membrane and actively transported out of the basolateral membrane by a calcium-dependent ATPase. Magnesium ion, another divalent cation of physiological significance, is passively absorbed by simple diffusion or convection, in contrast to calcium. Iron is absorbed in the ferrous form with the aid of specific cytoplasmic and membranous iron-binding proteins.

B.2.2 Carbohydrates

The monosaccharides glucose, galactose, and fructose are the final products of luminal digestion of carbohydrates. These compounds are water-soluble but are too large to be passively transported through the mucosal membrane. Glucose and galactose are actively transported through the brush border membrane by a membranous carrier protein that binds the sugar molecules and sodium ion. In this way, movement of sodium down the concentration gradient between the intestinal lumen and the enterocyte is available as a source of energy for active transport of glucose or galactose up a concentration gradient.

Active transport is generally not necessary in the upper jejunum, where the concentration of nutrients after a meal is high relative to the concentration inside of the enterocytes. As a result, in this part of the intestine the sugar-sodium carrier serves to merely facilitate diffusion along a downhill concentration gradient. As the chyme passes along the intestine, however, the absorption of sugars reduces the luminal concentration below that inside the enterocyte and active transport becomes necessary to remove the last portions of carbohydrates from the lumen.

In contrast to glucose and galactose, fructose is transported across the brush border by facilitated diffusion rather than by active transport. The fructose carrier is not linked to sodium ion transport or any other source of energy. Monosaccharides diffuse out of the basolateral side of the enterocyte by facilitated diffusion.

B.2.3 Proteins

Proteins are digested to tripeptides, dipeptides, and amino acids. These components enter the enterocyte by the same mechanism as monosaccharides. Several carrier proteins in the brush border membrane specifically bind the small peptides and amino acids along with sodium, so that they can be actively transported into the enterocyte. Four different carriers of amino acids have been identified which transport a total of 23 different amino acids. These carriers are absolutely specific for the L-stereoisomers of amino acids, but are less discriminatory of other structural variations, since each carrier transports several different amino acids. The characteristic groups recognized by the four carriers are neutral, dibasic, dicarboxylic, and imino structures. The simplest amino acid glycine is transported by both the neutral and the imino carrier. Peptides that are transported into the enterocyte are hydrolyzed to amino acids which diffuse out of the enterocyte through the basolateral membrane.

B.2.4 Lipids

Because of their insolubility in water, the absorption of dietary lipids presents a unique challenge to the intestinal mucosa. Triglycerides are the predominant dietary lipid, with phospholipids and cholesterol esters as the other major water-insoluble dietary components. The digestion of these compounds results in the formation of mixed micelles, which are aggregates of free fatty acids, monoglycerides, lysophospholipids, and free cholesterol together with bile salts. The mixed micelles are able to penetrate the unstirred layer of water and the mucous coat which covers the brush border surface of the lumen. These two structures present the major barrier to the passage of water-insoluble compounds into the enterocyte. Once the micelles have passed through the unstirred layer and the mucous coat, the micelle contents, with the exception of bile salts, dissolve readily in the hydrophobic phase of the brush border membrane and enter the enterocyte. The bile salts remain in the lumen in micellar form, available for mixed micelle formation with additional products of lipid digestion.

Having entered the enterocyte, the free fatty acids, monoglycerides, lysophospholipids, and cholesterol are resynthesized into triglycerides, phospholipids, and cholesterol esters by enzymes of the endoplasmic reticulum. Droplets of these lipids are packaged within the enterocyte by coating its

surface with proteins and phospholipids to form stable, insoluble droplets of lipid known as chylomicrons. The chylomicrons are packaged in secretory vesicles which fuse with the basolateral membrane, resulting in the release of the chylomicrons into the submucosa. The chylomicrons diffuse into the lacteals, where they enter lymphatic circulation.

A different mechanism of absorption is available for shorter chain fatty acids, less than 9 carbons in length. These compounds are not acted upon by the enzymes that synthesize triglycerides and are sufficiently water soluble to diffuse through the enterocyte and into blood capillaries without reincorporation into triglycerides or chylomicrons. Thus they enter the portal circulation. Fatty acids of intermediate chain length, 10 to 14 carbons, are absorbed from the intestine by both pathways.

B.2.5 Vitamins

With the exception of vitamin B6, which is transported by simple diffusion, the common water-soluble vitamins are transported by specific carrier proteins. The lipid-soluble vitamins A and K are transported with the aid of specific carriers, while vitamins D and E, which are also lipid-soluble, exit the lumen by simple diffusion. All of the lipid-soluble vitamins require the digestion and emulsification of accompanying dietary lipid for efficient absorption. This implies a role for lipid micelles in the solubilization and presentation of these compounds to the brush border membrane.

APPENDIX C

LIST OF HALOGENATED HYDROCARBONS SURVEYED IN PHASE II

- Trichloroethylene
- Methylene Chloride
- Chloroform
- Tetrachloroethane (1,1,2,2)
- Tetrachloroethylene
- Carbon Tetrachloride
- Trichlorofluoromethane
- Chloroethane
- Vinyl Chloride
- Dichloroethane (1,1 and 1,2)
- Dibromomethane
- Dibromochloromethane
- Dichloroethylene (1,2 and 1,1)

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